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THE BOTANICAL GAZETTE

February 1912

Editor: JOHN M. COULTER

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VOLUME LIII NUMBER 2

THE
BOTANICAL GAZETTE

FEBRUARY 1912

THE LIBERATION OF HEAT IN RESPIRATION

GEORGE J. PEIRCE

(WITH EIGHT FIGURES)

In September 1908, the BOTANICAL GAZETTE[1] published an account of some preliminary experiments, of a qualitative sort, in which I had used silvered Dewar flasks as respiration calorimeters. Continued experimenting with these flasks, and the reports of correspondents, have further confirmed their usefulness in physiological laboratories. They have also led me to doubt whether, after all, a study of the heat yield alone will lead one very far toward a solution of the problems presented by respiration.[2] In this paper, however, I propose to give the data of some of my experiments, continuing and extending the work previously reported, and after that to discuss the possible significance of the results.

The quantity of heat liberated by germinating peas

In the preliminary paper already referred to, my experiments were necessarily rough, in accuracy below the efficiency of the apparatus, which, however, was used under laboratory conditions and not under constant temperature. The necessity of working in a constant temperature is apparent, when any attempts to determine heat yields is being made, since the experiments continue through several days. Constant temperature rooms are generally hard to secure and hard to keep clear of fungus spores, bacteria, etc.

[1] BOT. GAZ. **46**: 205–220. 1908.

[2] Plant World **12**: 193–198. 1909.

The Botanical Department of this University has been particularly fortunate in falling heir to two large chambers, the temperature of which has been remarkably constant during the months in which temperature records have been kept. These rooms are underground, built of concrete, and were used at one time as tanks in which fuel oil was stored. With a change in the means of heating our building, these tanks ceased to be used. The site of the experiment house, which was built in the summer of 1908, was so selected that these two tanks came under the floor of the greenhouse. The tanks were then cleaned of the residual fuel oil, and were ready for use as constant temperature chambers when the house over them was completed. Each is entered through a manhole in the floor of the greenhouse, the descent being over an iron ladder. Walls, floor, roof, and ladder are of such material that they may be washed or hosed off with an antiseptic solution, which may then be quickly swabbed up. The air, never excessively dry, can easily be maintained at any degree of humidity that one chooses, by occasionally sprinkling the walls. The following table will indicate the temperatures in one of these tanks during a period of nearly five months, these temperatures being taken by a maximum-minimum thermometer of the United States Weather Bureau pattern.[3] They are as follows:

Date	Time	Room	Maximum	Minimum	Differences
March 26....	.....	16.4°C.	.....	.....	.....
March 28....	10:00 A.M.	16.25	17.22°	16.67°	0.55°
March 29....	9:30 A.M.	16.2	17.11	16.61	0.50
March 30....	.....	16.2	17.11	16.61	0.50
March 31....	.....	.....	17.05	16.55	0.50
April 1.....	.....	.....	17.22	16.55	0.67
April 2.....	.....	.....	17.22	16.50	0.72
May 12.....	.....	.....	18.89	17.78	1.11
May 13.....	.....	.....	18.89	18.05	0.34
May 14.....	.....	.....	18.61	18.05	0.56
May 15.....	.....	.....	18.61	18.05	0.56
May 16.....	.....	.....	18.83	18.05	0.78
May 17.....	.....	.....	18.77	17.73	0.94
May 18.....	.....	.....	19.07	17.91	1.06
August 13....	10:30 A.M.	20.5	20.55	17.78	....

Inspection of this table shows that the lowest maximum was on March 31, a temperature of 17.05° C. The lowest minimum was

[3] Bausch & Lomb Optical Co.'s Catalogue, no. 17,004.

two days later, April 2, a temperature of 16.50° C. The highest maximum came at some time during my absence in the summer vacation, a temperature of 20.55° C., which, however, differed from the temperature at the time I made and recorded the observation on August 13 by only 0.05°. The highest minimum was in mid May. Between the highest and lowest maxima there was a difference of 3.5°; between the highest and lowest minima a difference of 1.55°; and between highest maximum and lowest minimum a difference of 4.05° from late March to mid August, with an average daily range of 0.71° on 13 days in March, April, and May. It is needless to say that these chambers might be warmed, and presumably their temperatures would be very nearly as constant, but in this mild climate, in which there is little frost even at night in midwinter, and still less excessive heat in midsummer, artificial heat seems unnecessary, at least for experiments on the respiration of such germinating seeds as peas.

Having thus established the remarkable constancy in the temperature of these chambers, I may now pass to a consideration of the apparatus employed. I have used silvered Dewar flasks made by Burger of Berlin and imported "duty free." These Burger-Dewar flasks are stamped and numbered by the maker. Their efficiency is very uniform. Experience with the unsigned flasks of other makers quickly shows that they are not so uniformly efficient as they should be. For the sake of convenience and economy, not at first realizing my mistake, I have continued to use flasks of about 250 cc. capacity. But I am sure that flasks of not less than double this capacity would be more satisfactory because involving smaller physical errors.[4] Most of the flasks are simply double-walled round-bottom flasks, like fig. 1, but I have had four made in which, as indicated by fig. 2, the interior drains through a tube opening at *a*. This opening is small, but it seems to be sufficient to carry off carbon dioxide as well as water. At the same time, it does not greatly reduce the efficiency as an insulator of a well-made flask, and does add materially to its convenient use, making it possible to

[4] See OSTWALD-LUTHER, Physikalisch-chemische Messungen **2**:189, 1902, where BERTHELOT's results are cited as indicating that it is not practicable to use calorimeters of less than half-liter capacity.

soak seeds in the flask, and then, when one is ready, to draw off the water without exposing or disturbing the other contents. The efficiency of silvered double-walled Dewar flasks used as insulators is indicated by the following experiment. Four flasks, numbered 1, 3, 4, and 6, held in pairs in the wooden filter stands usual in chemical laboratories, were set on a table in my laboratory. Into each were introduced 140 cc. of hot, distilled water; a long thermometer reading to tenths of a degree was placed in each flask and held, with the bulb almost touching the bottom, by a tight plug of absorbent cotton placed in the neck. The successive thermometer readings and other data follow:

FIG. 1

FIG. 2

FLASK	AUGUST 26, 1909				AUGUST 27, 1909	SEPTEMBER 1, 1909
	11:30 A.M.	1:45 P.M.	3:05	5:05	11:05 A.M.	2:15 P.M.
1.....	72.4°C.	67.05°	64.4°	60.8°	41.0°	21.4°
3.....	73.1	61.75	56.7	50.6	27.4	21.0
4.....	60.05	54.0	51.12	47.5	Hg col. separated	.....
6.....	63.4	57.6	54.74	51.0	20.5	20.75
Room.....	21.11	20.75	21.2	20.75	20.5	20.75

A similar experiment is recorded in the following table, in which drained flasks were used in constant temperature room A:

FLASK	APRIL 18, 1910		APRIL 19, 1910	
	11:04 A.M.	12:05 P.M.	12:20 P.M.	4:15
11–5.....	68.7°	65.6°	32.4°	30.2°
12–2.....	72.7	69.9	38.6	36.25
14–6.....	61.1 (broke)	46.9	17.8	17.8
8–8.....	51.15	49.9	30.7	28.9
Room.....	17.0	17.5	17.3	17.3

The graphic representation of these figures, as in the accompanying curves (figs. 3 and 4), indicates more plainly the different degrees of efficiency as insulators possessed by different flasks, even of the same manufacture. The record of flask 14 with thermometer 6, in fig. 4, shows very strikingly the part played by the vacuum as an insulator between the two silvered walls of these

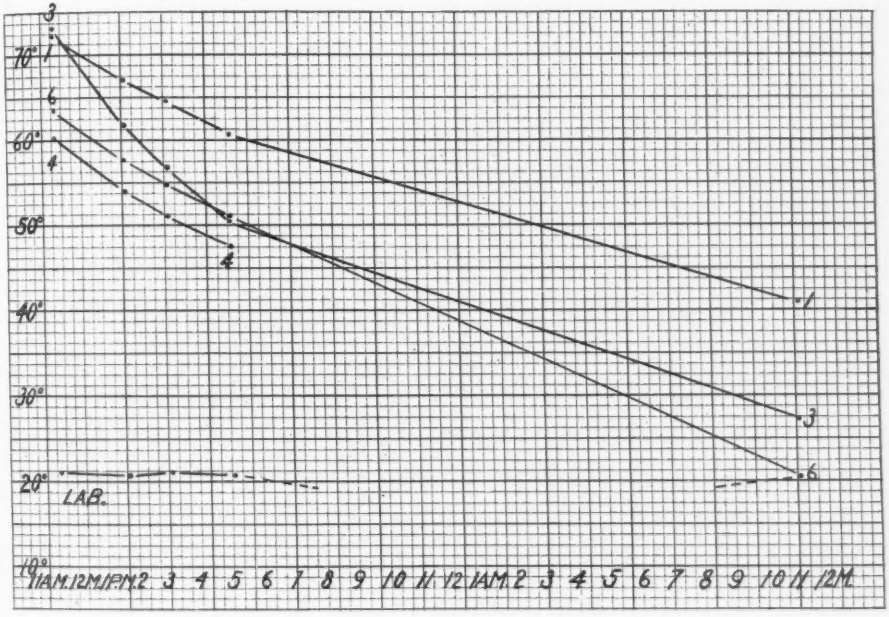

FIG. 3.—Graphic representations of temperatures in silvered Dewar flasks and in the laboratory.

double-walled flasks, for I cracked this just after pouring in the warm water. Although I kept the water in the inner flask, the vacuum surrounding it was destroyed. The temperature fell to approximately that of the room, almost as rapidly as in a single-walled unsilvered vessel. For obvious reasons, the rate of fall in temperature is most rapid when there is the greatest difference in the temperatures within and without the flask. This fact is impor-

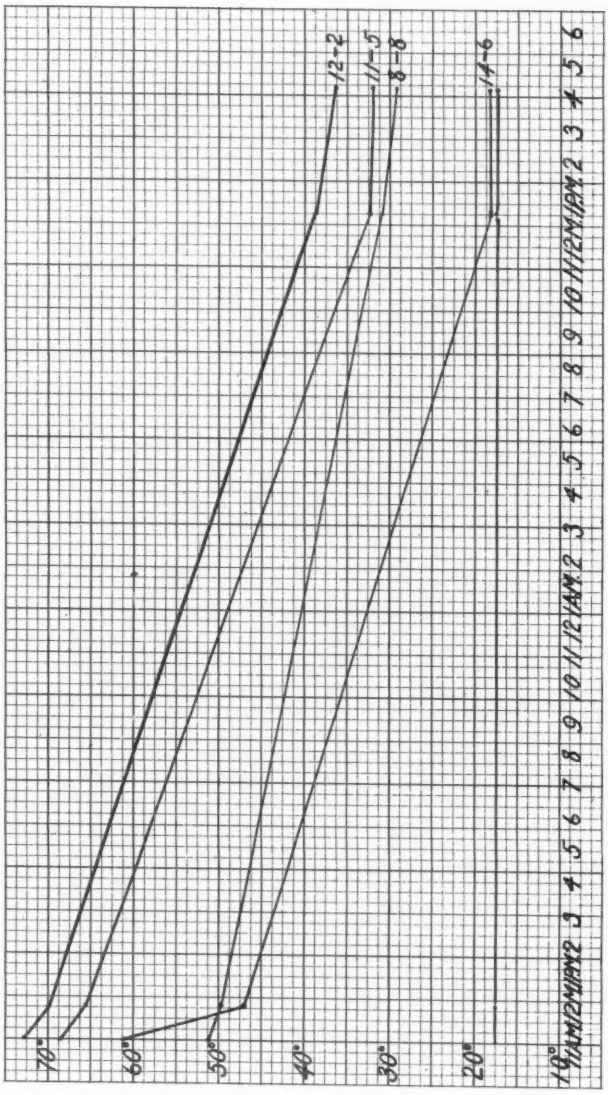

FIG. 4.—Graphic representations of temperatures in silvered Dewar flasks and in the constant temperature room

tant in connection with respiration experiments in which heat production is being studied; for evidently the flasks, as well as the organisms within them, will lose heat most rapidly through the air when the air is decidedly cooler than they are, whether because the air is actually cold, or because the heat liberated by the organisms in the flask accumulates. The total heat liberated, therefore, must be the sum of the heat lost through radiation, etc., plus that retained by the flask. By placing the flasks in a constant temperature, the rate, and therefore the amount, of heat-loss can be determined for each flask and each degree of difference in temperature.

Another factor to be reckoned before we can attempt even approximately to determine heat yields is the heat equivalent of each combination of apparatus. This work has revealed to me, as I never suspected before, the limitations of the ordinary thermometer as an instrument of precision. In the following determinations, as will be seen below, I have used the same thermometers and the same cotton plugs in the same flasks throughout the series of experiments, thus determining the heat equivalents of each calorimeter, consisting of thermometer, Dewar flask, and plug. Indeed, I kept the flasks in the same places on the wooden filter stands and even on the same spot on my table. These latter precautions are, however, strainings at gnats, for the camel of unavoidable error to be swallowed is very large, owing to the small size of the flasks.

The method followed to ascertain the heat equivalent of each set of apparatus was fundamentally as follows, although I modified one detail or another in the series of determinations which I attempted. Into a flask, the temperature within which is known and recorded, 200 cc. (100 cc. if the flask already contains 100 cc.) of warm distilled water, at a known temperature, are *quickly* poured; the temperature within the flask is again recorded, as soon as it has again become fairly stable. The fall in the temperature of the water poured into the flask indicates that the flask and the inclosed air, thermometer, and cotton plug, have taken up warmth from the water. If all the water could be delivered into the flask at the temperature recorded, the fall in temperature would indicate only the amount of heat taken from the water by the apparatus. But this ideal condition I have found practically unattainable.

Working with only 200 cc. of water, at the most, I have never been able to pour this water from the flask in which it was heated into the Dewar flask so quickly that no heat was lost. I used a thin-walled round flask for heating the measured quantity of water; I cut the neck of the flask to a length of 2 cm.; while being heated the flask was held by a small wooden test tube holder of the usual form; the thermometer for determining the temperature of the water remained with its bulb under water in the flask while it was heated; and I took pains not to let the flask touch anything after taking it from the flame, until it was emptied. By holding the flask with the wooden holder while the water in it was warming, I warmed the holder, and as wood is a non-conductor, I felt that I was taking the least possible heat from the water myself in pouring it. In these and in other ways, therefore, I have taken such pains as I could to reduce the inevitable error to its lowest terms; but the error in calorimetry is stated by LUTHER-OSTWALD to be about 1 per cent under the most favorable conditions, and with larger volumes of liquid than I have so far been able to use. A typical series of figures follows, with the averages of a considerable number of determinations for four calorimeters.

April 2, 1910	No. 11–5	No. 12–2	No. 14–6	No. 8–8
Temperature, empty	27.6°	29.2°	29.1°	28.7°
Add 200 cc	45.0	42.0*	42.0	40.
Becomes	43.2	40.75	40.5	38.55
Loss	1.8	1.25	1.5	1.45
Calories lost	360.0	247.0	300.0	290.0
Gain temperature	15.6	11.55	11.4	9.85
Calories per degree	23.07	21.4	26.3	29.4

* Measured 197 cc.

The averages for four calorimeters follow:

No. 11–5, 15 determinations average 24.7 calories per °C.
No. 12–2, 15 " " 24.4 " " "
No. 14–6, 10 " " 24.8 " " "
No. 8–8, 13 " " 28.5 " " "

The average of these four calorimeters is 25.6 calories per Centigrade degree. This means that the average calorimeter, consisting of a silvered Dewar flask of 250 cc. capacity made by Burger of Berlin, a long thermometer graduated to tenths of a degree, and a cotton plug suitable to close the neck of the flask, required a quan-

tity of heat represented by about 25 calories to warm its interior 1° C. Thus, if the temperature in one of these calorimeters goes up 10° C., about 250 calories had been taken by the apparatus itself. This is known as the "heat equivalent" of the apparatus, and is a quantity which must always be added or subtracted in determining the amounts of heat liberated or taken up by a given organism, process, or reaction.

In addition to the heat lost by radiation, etc., from the insulating calorimeter, and the heat absorbed by the whole apparatus (flask, thermometer, and plug) and the air contained by it, the material within the flask will also absorb heat. It is necessary, therefore, to determine the heat equivalents of the material worked with, whether these materials are seeds or solutions. The solutions may contain nutritious substances in which such organisms as bacteria, yeasts, or fungi are living. In any case, the heat equivalents of all the substances or materials involved in the experiment must be ascertained. Thus, on one day I weighed out six lots of air-dry peas of 75 gr. each, putting these in crystallizing dishes, which I covered with glass lids and kept for 24 hours at a temperature of about 18° C. Six Dewar flasks with thermometers and plugs, and containing some distilled water, were also placed under the same condition. After 24 hours I drained (but did not dry) the flasks, and put one lot of peas in each, reading the temperature of each. I assumed that the peas had in this time taken on a uniform temperature before being put into the flasks. The slight differences in temperature, therefore, were presumably due to slight differences in the temperatures of the flasks and to slight differences in the amounts of heat due to friction in pouring the air-dry peas into the flasks. Then I poured 100 cc. of warm distilled water at a known temperature into each flask and, when the temperature had become stable, recorded it. The data are as follow:

Flask	Therm.	Temp.	100 cc. aq.	Becomes	Loss
1.....	1	19.7°	57.0°	45.3°	1170 cals.
2.....	2	19.2	55.5	43.5	1200
3.....	3	19.3	56.0	44.2	1180
4.....	4	19.5	54.0	43.0	1100
5.....	5	19.8	52.0	41.2	1080
6.....	6	19.8	49.5	40.4	910

From these figures and from the previous determinations of the average number of calories required to raise each set of apparatus one degree in temperature, I calculated the number of calories required to raise 75 gr. of air-dry peas 1°. These figures and the average follow:

To raise 1° requires:

1 = 45.70	calories	less	average	no.	calories	required	to	raise	1 − 1° − 11.62 = 34.08c
2 = 49.38	"	"	"	"	"	"	"	"	2 − 1° − 13.25 = 36.13c
3 = 47.78	"	"	"	"	"	"	"	"	3 − 1° − 12.81 = 34.97c
4 = 46.80	"	"	"	"	"	"	"	"	4 − 1° − 13.49 = 33.31c
5 = 50.46	"	"	"	"	"	"	"	"	5 − 1° − 11.67 = 38.79c
6 = 44.17	"	"	"	"	"	"	"	"	6 − 1° − 10.54 = 33.73c

Total	211.01c
Average to raise 75 gr. air-dry peas 1°	35.16c
" " " 1 gr. " " 1°	0.4688c

From this it appears that it takes 0.468 of a calory to raise one gram of air-dry peas 1° in temperature. A repetition of the experiment with the same apparatus and under similar conditions gave an average of 0.457 of a calory per gram of air-dry peas.

The same experiment was repeated on a later date with this modification; 6 lots of air-dry peas weighing 75 gr. each were put in the same 6 Dewar flasks and washed in a concentrated aqueous solution of mercuric bichloride. After rinsing twice with boiled distilled water, the peas were covered with 100 cc. of boiled distilled water in each flask, thermometers and cotton plugs were inserted as usual, and the whole allowed to stand for 24 hours in the laboratory. The temperatures in the flasks and in the room at the hours stated were as follow:

Flask	March 10, 1909			
	12:00 M.		2:00 P.M.	
1	16.0°	Room 18°	16.55°	Room 20.5°
2	16.0	.....	16.50	.....
3	16.0	.....	16.70	.....
4	16.8	.....	17.20	.....
5	17.5	.....	18.10	.....
6	17.5	.....	18.05	.....

The next day the temperatures were recorded as below, and 100 cc. of warm distilled water at the temperatures reported were poured into each flask. Flask 5 exploded at this stage. The following figures indicate the heat equivalents of the five remaining lots of peas, and these yield an average of 1.41 calory per degree for 75 gr. of peas weighed air-dry and soaked for 24 hours in 100 cc. of boiled distilled water. This figure would be the sum of the heat equivalent of pea substance weighed air-dry and water, if no change had taken place in the substance of the peas during this interval of 24 hours in water; but assuming, for the moment at least, the correctness of the figure 1.41 calory per degree, one must realize that in this time germination had begun, and that the materials in the peas had begun to undergo changes, not only from absorption and the solution or dilution of the soluble substances in the peas, but from digestion and other metabolic processes. The figure 1.41 calory per degree, therefore, may be correct or approximately so, for peas which had been for 24 hours under conditions favorable to germination, and hence were not necessarily identical in composition with air-dry peas of the same variety.

MARCH 11, 1909, 9:30 A.M.; ROOM 15.75°

1.....	18.7°	100 cc. aq. 64.3°	Becomes 39.12°	Loss 2518 cal.
2.....	18.4	56.4	35.9	2050
3.....	18.4	60.2	37.8	2240
4.....	19.4	58.5	37.6	2090
5.....	19.3	Exploded		
6.....	19.4	56.0	35.7	2030

To raise 75 gr. peas soaked 24 hrs. *in situ* 1° requires:

1	=	123.11		calories less average for flask 1 and therm.				1	=	11.62	= 101.49 cal.
2	=	116.42	− 13.25	"	"	"	"	2	"	"	= 103.17 "
3	=	114.87	− 12.81	"	"	"	"	3	"	"	= 102.06 "
4	=	114.83	− 13.49	"	"	"	"	4	"	"	= 101.34 "
6	=	123.78	− 10.54	"	"	"	"	6	"	"	= 113.24 "
Average for 75 gr. peas soaked per degree											= 106.26 "
" " 1 " " " " "											= 1.41 "

I think we are now ready to proceed to ascertain the heats liberated by peas from the beginning of germination until it has progressed for several days. An experiment was set up as follows:

March 26, 11:30 A.M.; room temperature 16.4° C. Five lots, 75 gr. each, of air-dry peas were kept in constant temperature room A for a week, in five Dewar flasks. Thermometers, bottles of boiled distilled water, and of saturated aqueous solutions of mercuric bichloride, had been placed in room A at the same time, and were therefore of like temperature. Each lot of peas was then thoroughly washed in the bichloride solution, rinsed twice with boiled distilled water, and covered with 100 cc. of boiled distilled water in each flask. (The rinsing water came from the laboratory and had a temperature 2° C. higher than that put in the flasks to start germination.)

The temperatures were:

Flask 1, therm. 1, 17.4°
" 2 " 2, 17.3
" 3 " 3, 16.9
" 4 " 4, 18.4
" 6 " 6, 17.5

MARCH 27, 10 A.M.; ROOM 16.25°

1.....	18.9°	−37.0 cc.	Absorbed 63.0 cc. aq.
2.....	18.7	−44.0	" 56.0
3.....	17.55	−40.5	" 59.5
4.....	18.3	−45.4	" 54.6
6.....	18.4	−45.5	" 54.5

I poured off the unabsorbed water in each flask as indicated above, showing that each lot of 75 gr. of air-dry peas had absorbed the number of cc. of water above stated. The rise in temperature in each flask showed the liberation of heat. Reckoning the heat equivalents already found of the pieces of apparatus, the peas, and the water, we find that there were liberated in each flask the following quantities of heat, plus whatever had been lost by radiation through the flasks, by leakage through the cotton plugs, etc., in the first 22.5 hours of the experiment, namely:

1.....	1.5°	218.55 calories
2.....	1.4	96.00
4.....	0.00	0.00
6.....	0.9	129.75
Average ..	.....	130.68 calories

MARCH 28, 10 A.M.; ROOM 16.25°

	No.	Temp.	Gain in 24 hrs.	Total gain
1	23.8°	4.9°	6.4°	.....
2	24.7	6.0	7.4	.....
3	20.4	2.85	3.5	.....
4	21.2	2.9	2.9	.....
6	22.1	3.7	4.6	.....
Average	.....	4.07	4.96	482.71

MARCH 29, 9:30 A.M.; ROOM 16.2°

1	32.3°	8.5°	14.9°	.....
2	38.55	13.85	21.25	.....
3	25.00	4.6	8.1	.....
4	25.9	4.7	7.6	.....
6	29.4	7.3	11.9	.....
Average	.....	7.79	12.95	923.90

MARCH 30, 9:00 A.M.; ROOM 16.2°

1	39.0°	6.7°	21.6°	.....
2	45.1	6.55	27.80	.....
3	30.25	5.25	13.35	.....
4	31.8	5.9	13.5	.....
6	36.7	7.3	19.2	.....
Average	.....	6.34	19.09	751.93

MARCH 31, 1909, 9:30 A.M.; ROOM 16.1°

1	40.9°	1.9°	23.5°	.....
2	50.8	5.7	33.5	.....
3	32.6	2.35	16.7	.....
4	32.3	0.50	14.0	.....
6	37.25	0.55	19.75	.....
Average	.....	2.20	21.49	260.92

APRIL 1, 1909, 9:30 A.M.; ROOM 16.4°

1	41.3°	0.4°	23.9°	.....
2	52.55	1.75	35.25	.....
3	33.1	0.5	17.2	.....
4	30.7	−1.6	12.4	.....
6	34.8	−2.45	17.3	.....
Average	.....	−0.28	21.21	−33.16

APRIL 2, 1909, 9:30 A.M.; ROOM 16.5°

1.....	44.3°	3.0°	26.9°	.....
2.....	53.3	0.75	36.0	.....
3.....	33.8	0.7	17.9	.....
4.....	30.6	−0.1	12.3	.....
6.....	33.7	−1.1	16.2	.....
Average.....	.....	0.65	21.86	77.09

At this point the experiment was stopped and the flasks emptied. The peas in no. 4 showed practically no molding or fermentation. The odor was entirely pleasant, and the radicles of these germinating seeds were long and fine. Flask 2 was found to contain very rotten peas; the peas in flask 1 were fairly rotten; in flasks 3 and 6 the peas were about equally bad, but decidedly less rotten than in 1. This result agrees with the experience of HANNIG, who says: "Nach Erfahrungen des Referenten kann eine Sterilisirung von Samen durch Schütteln in Sublimatlösung alleine nicht erzielt werden, sondern nur dadurch, dass die Samen zuerst mit verdünntem Alkohol oder ausgekochtem Wasser behandelt werden, um die an den Spalten der Samen haftenden Luftblasen zu entfernen."[5] Only experience led me to believe in the possibility of sterilizing by HANNIG's method. Owing to the long duration of these experiments, complete sterilization becomes as difficult as it is desirable. I find no help in this particular in SCHROEDER's recent paper,[6] although for other plants and for briefer times his directions are most welcome.

Adding together the daily gains in heat, we find that, up to the sixth day, on which more heat was lost by radiation than gained in respiration, there had been liberated 2550 calories which had not been lost or converted into work. On the sixth day 33.16 calories more were lost in radiation (or otherwise) than had been liberated during that time in respiration. But when the experiment was stopped on the seventh day, this loss had been made up, and there had been an average total gain of 2593.63 calories in each flask, after all corrections for absorption of heat by the materials con-

[5] Rev. in Zeitschr. Bot. 1:202. 1909.

[6] SCHROEDER, H., Die Wiederstandsfähigkeit die Weizen- und Gerstenkorns gegen Gifte und ihre Bedeutung für die Sterilisation. Centralbl. Bakteriol. 2:28. 1910.

cerned had been made. This amounted to a gain of 370 calories per day, when the seven days are averaged, or 4.93 calories per day for each gram of peas. The comparative daily changes in room temperatures, flask temperatures, and the number of calories liberated, are shown on the accompanying diagram (fig. 5).

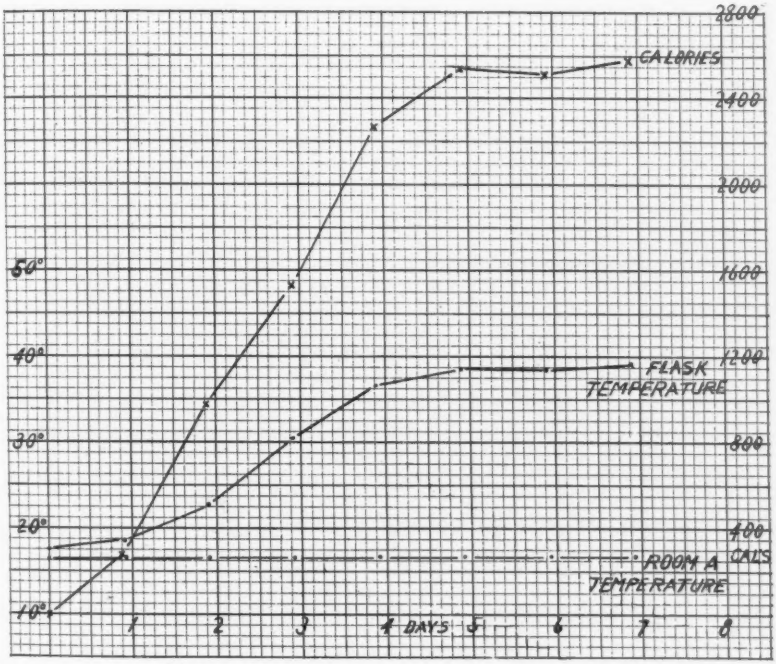

FIG. 5.—Graphic representation of the amount of heat liberated by germinating peas

A comparison of these figures with those of BONNIER'S classical paper[7] shows at once that my figures are lower than his. For example, he says (p. 12), "1 kilogramme de graines germant dégage en 1 minute 59 calories." The greatest average gain in heat in my experiment was on the fourth day, in which 923.9 calories were accumulated in 23.5 hours. Calling this 924 calories and 24 hours,

[7] BONNIER, G., Recherches sur la chaleur végétale. Ann. Sci. Nat. Bot. VII. 18:12. 1893.

we get 8.55 calories per minute for a kilo of peas. But one sees at once that in BONNIER's experiments only short intervals of time were employed (36 minutes for the above quoted experiment), whereas my experiments lasted for nearly or quite one week. In fact, I made no effort to "break the record," and was not aware of the record until I figured my results in order to compare them with BONNIER's. I venture to think, however, that my experiments supplement those of BONNIER by showing the continued liberation and loss of great quantities of heat by germinating peas, and presumably by other plants or their parts, for a time and not merely minute by minute. I need hardly say that the experiment above described in detail is only one of many, the results of which are so similar that it is unnecessary to report them.

The quantity of heat liberated by certain animals

Having some curiosity about the liberation and loss of heat by animals, as compared with plants, I made the following experiments. One was with two salamanders lent me by the Department of Physiology of this University. I put them in separate drained Dewar flasks, and left them for 24 hours in the laboratory. The temperature in the flasks fluctuated with the room temperature, so nearly in the same way as if no animals had been in the flasks, that I did not continue the experiment. I report it with no further comment than this, namely, that the salamanders must have exhaled some warmth, but it was inappreciable.

The other experiment was very striking. Into cotton-plugged drained Dewar flask 14, which weighed 167.2 gr., I put a very lively mouse. The weight at the end of the experiment was 186.5 gr.; therefore, the mouse weighed 19.3 gr. As no excretion, solid or liquid, had taken place during the experiment, whatever loss of weight had occurred was due to exhalation and evaporation only. The thermometer readings for a half hour, beginning at once after putting the mouse in the flask, were as follow, the room temperature remaining stationary at 20° C.

During this half-hour of time, therefore, this mouse gave off by radiation and exhalation an amount of heat sufficient to raise the temperature within the apparatus 13.95° C. As previously stated,

the average of 10 determinations shows that it takes 24.8 calories to raise the temperature of flask 14, with thermometer 6 and a cotton plug, 1° C. Hence the heat *given off* by this mouse in 30

Time	Temperature	Time	Temperature
2:30 P.M.	22.6°	2:47.5 P.M.	32.0°
2:34	23.1	2:48	32.2
2:35	24.3	2:48.5	32.4
2:35.5	24.6	2.49	32.6
2:36	25.1	2.49.5	32.7
2:36.5	25.4	2:50	32.9
2:37	25.7	2.50.5	33.1
2:37.5	26.2	2.51	33.2
2:38	26.4	2:51.5	33.6
2:38.5	26.7	2.52	33.9
2:39	27.1	2:52.5	34.1
2:39.5	27.5	2:53	34.5
2:40	27.9	2:53.5	34.8
2:40.5	28.3	2:54	35.0
2:41	28.7	2:54.5	35.2
2:41.5	29.0	2:55	35.35
2:42	29.2	2:55.5	35.5
2:42.5	29.4	2:56	35.6
2:43	29.6	2:56.5	35.7
2:43.5	29.9	2:57	35.9
2:44	30.1	2:57.5	36.05
2:44.5	30.5	2:58	36.3
2:45	30.7	2:58.5	36.1
2:45.5	31.0	2:59	36.2
2:46	31.2	2:59.5	36.4
2:46.5	31.5	3:00	36.55
2:47	31.7		

minutes amounts to 345.96 calories at least. This takes no account of the amount of heat lost in this time by this set of apparatus, being given off to the air; nor does it include any estimate of the heat retained by the body of the mouse. The body temperature of a mouse I do not know, nor do I know whether the body temperature rose during the experiment. This amount of heat, therefore, is simply what is absolutely lost to the animal, an amount equal to 11.19 calories per minute. The rise in temperature is indicated by fig. 6.

Does the age of the seed affect the heat yield?

I do not know that any successful attempt has been made to answer this question, in spite of the theoretical and practical importance which are evident on a moment's reflection. If the

heat yield bears a fairly uniform relation to the age of the seed, this may be the cause, or it may merely be the reflection or symptom or result of that chain of causes, which finally deprives the seed of its germinating power. Even so, it is important to determine whether this is the case or not. And if it can be shown that the

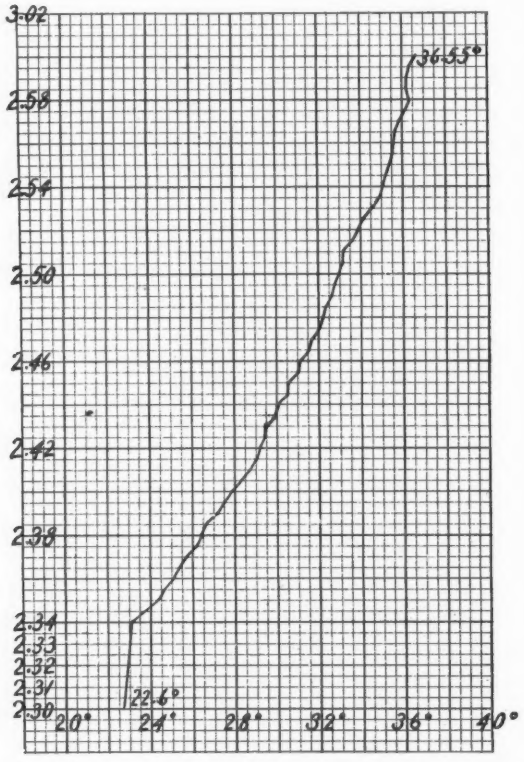

FIG. 6.—Rise in temperature in flask 14, in which a mouse was confined

heat yield is proportional, or inversely proportional, to the age of the seed, other things being equal, and a convenient method can be employed for determining this, we shall have, for the first time, a means of ascertaining the age and the viability of seeds. Those who have had any experience in buying seed have also known the

disappointment in fine-looking but worthless seed which failed to germinate. The Pure Food and Drugs Act has made it necessary to take fewer articles which come under its operation on faith or on the word of the dealer. But there being no convenient way of testing the quality of seeds, they are as unreliable as ever. It is highly desirable, therefore, from a practical standpoint, to have a method of testing seeds as to their germinating power. Such a method must be reasonably accurate and convenient. I believe it will be found that Dewar flasks may be adapted to this use, and I wish to furnish a certain amount of evidence now in favor of this belief, hoping to be able to determine the matter before long.

Peas retain their germinating power much longer than many other seeds, longer than the ordinary grains, for example.[8] They are even less suitable, therefore, for experimental investigation of this matter than many other seeds. I happen to have in my laboratory, however, peas bought in 1908, 1910, and 1911. Each lot must have been of the preceding year's crop, or older; but owing to the large yearly demand for pea seed, probably very little old stock of this sort is carried over, and I believe, therefore, that the seeds in my laboratory are, most of them at least, of the crops of 1907, 1909, and 1910. The variety is commercially known as "American Wonder," an "extra early." The following table shows the behavior of these three crops in Dewar flasks this year, in constant temperature room A. The flasks and thermometers were sterilized by washing with concentrated aqueous solution of corrosive sublimate, and subsequently rinsed three times with sterile distilled water. The peas were weighed, air-dry, in 80 gr. lots and put, with 100 cc. boiled distilled water, in each of the flasks to be used. The boiled water and the peas, as well as all the parts of the apparatus, were kept in the constant temperature room for at least 24 hours before an experiment was set up. The water was drawn off at the end of the first day.

To this table I should like to add the figures reported in my first paper describing the use of Dewar flasks as respiration calorimeters,[9] for these figures were obtained by using the same 1907 crop of peas.

[8] PFEFFER, W., Pflanzenphysiologie 2^2: p. 327 and the literature there cited.

[9] PEIRCE, G. J., A new respiration calorimeter. BOT. GAZ. 46:199. 1905.

JANUARY 19–23, 1911; CROP OF 1910

		1st day	2d day	3d day	4th day	5th day
Flask 7.....	17.8°	18.7°	21.8°	27.8°	31.1°	....
Flask 8.....	17.8	18.5	22.3	26.9	31.5	....
Flask 13.....	17.7	18.5	22.4	26.4	33.0	....
Average gain		1.33	4.93	9.8	14.63	....

JANUARY 25–28, 1911; CROP OF 1909

Flask 9.....	17.0°	17.9°	22.7°	25.3°	....	....
Flask 10.....	17.5	18.4	23.7	26.4	....	....
Flask 11.....	17.1	19.0	23.1	24.9	....	....
Flask 12.....	17.0	18.0	24.2	27.9	....	....
Flask 13.....	17.0	19.5	25.15	28.1	....	....
Average gain		1.44	6.72	9.4	....	....

JANUARY 31–FEBRUARY 5, 1911; CROP OF 1907

Flask 10.....	17.2°	17.8°	21.8°	25.8°	29.1°	32.8°
Flask 11.....	17.1	17.8	19.4	23.7	23.6	25.2
Flask 12.....	17.1	17.6	19.3	21.3	23.2	24.4
Flask 13.....	17.2	17.8	22.2	26.8	30.8	35.4
Average gain		0.6	3.52	7.25	9.52	12.3

It should be said that the following temperatures were obtained under the far from uniform conditions of the laboratory, for I did not at that time have a constant temperature room.

APRIL 29–MAY 2, 1908; CROP OF 1907

Flask 1.....	22.7°	27.0°	33.4°	36.8°	....	....
Flask 2.....	21.6	25.7	35.1	40.5	....	....
Flask 3.....	22.1	27.3	35.3	41.4	....	....
Flask 4.....	22.4	27.4	35.0	38.8	....	....
Flask 6.....	22.3	26.5	34.5	40.0	....	....
Average gain.....		5.56	12.44	17.3	....	....

Assuming the correctness of these figures (and both the 1908 and 1911 figures above given are among many that might be given) and making all due allowances for the differences in temperatures between the laboratory and the constant temperature room, one must admit there appears to be a decided difference in the amounts

of heat given off. The flasks used in 1908 were inferior as insulators to those in use in 1911; hence the 1908 figures are not as high as they would have been if more efficient insulators had been employed. So far as these figures show, therefore, it appears that the amount of heat liberated by germinating peas decreases with

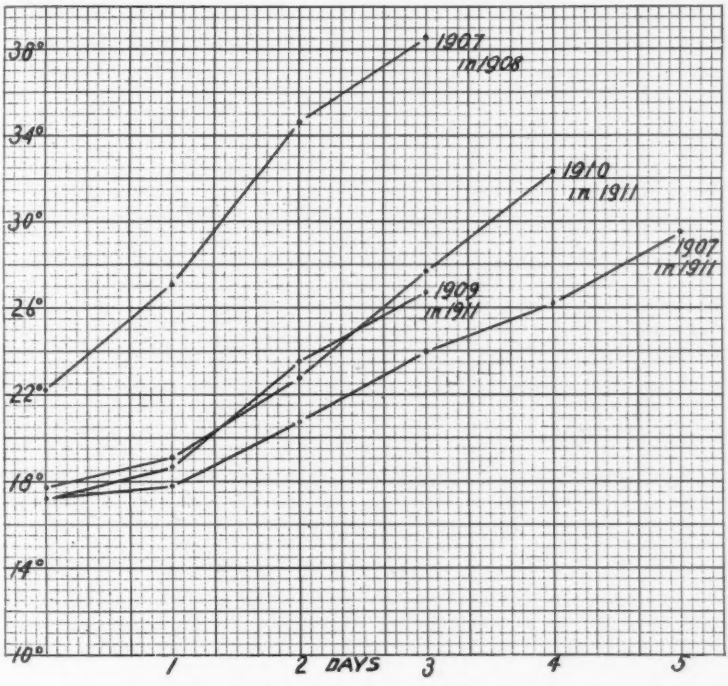

FIG. 7.—Heat yields of peas of different ages

their age. If this be true, the amount of heat liberated may then be used as the index of the age or freshness of seeds.

The accompanying diagram (fig. 7) represents the above figures in the form of curves. The difference between the curves of 1907 peas in 1908 and 1911 is even more striking than the figures on which they are based.

It does not by any means follow that the other processes of the

plant decline proportionally with the liberation of heat. Experience shows that old seeds germinate less well than fresh. The foregoing experiments show that the heat yield is less in old than in fresh peas. One record of mine, made up of many measurements, connects growth with these other two processes.

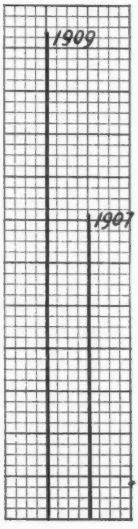

FIG. 8.—Lengths of seedling peas of same number of days' growth, but of different age of seed.

Peas which had been sprouted and kept for four days in Dewar flasks were subsequently put in moist sand to continue germination. The peas were of two lots, those of the 1907 crop and those of the 1909 crop. One week later they were carefully taken up and measured. The average length of the 1909 seedlings was 9.70 cm., of the 1907 seedlings 3.52 cm. The latter is scarcely more than 60 per cent of the former. The seedlings from the older seeds made less than two-thirds the growth of the seedlings from fresher seeds in the same length of time and under the same conditions. The accompanying diagram shows this graphically (fig. 8).

If the result just now described were not consistent with the difference in heat yields according to the age and freshness of pea seeds, I should place no reliance whatever on it. As it is, I do not understand why I did not test such an interesting result by repeated experiment, but I have not yet done so. The question seems to me to deserve further examination.

The possible significance of heat liberation in respiration

From the foregoing experiments, whether on germinating seeds or on warm-blooded animals, it appears that there is a much greater release of energy in the form of heat than can be or is used as such by the organism. Admitting that, under the extraordinary conditions of the experiment, the mouse previously described may not have behaved normally in any respect, and that the production and loss of heat may have been excessive, it is nevertheless evident

that the loss of heat by a warm-blooded animal is very great. When we reflect that all of the heat lost is liberated from the food of the animal, that the loss by radiation will vary with the temperature of the environment and the efficiency of the animal's covering as an insulator, whether this be hair, feathers, subcutaneous fat, or clothing, and that the loss by exhalation will also vary with the rate of exhalation, then we must realize that very much of the heat liberated in respiration is lost. What percentage this is in the case of a mouse I do not know, and it is hardly necessary to know at the moment. It is evident that if the liberation of heat is the essential result of respiration, respiration must be an excessively wasteful process.

Having once conceived respiration to be primarily for furnishing the living organism with energy in the form of heat, I am constrained now to regard it very differently. That it is the chief means of liberating energy still seems to me most probable; but apart from the production and maintenance of a body temperature apparently most favorable to the other functions collectively, the liberation of heat in respiration appears to be wasteful. It may be as useless as it is inevitable.

The material products of respiration vary with the food, with the materials oxidized, directly or indirectly, in the body. Intracellular respiration, or the catabolic processes, the end products of which are oxides and heat, may not only produce within the cell the optimum temperature for its activities and liberate energy at once converted into work, but they may also neutralize (destroy) substances which would otherwise be or become injurious to it. The oxidation of these substances would necessarily be accompanied by heat liberation. The heat thus liberated must be either converted into work or given off, lest again the organism suffer. We may, therefore, conceive the heat liberated in respiration to be of two sorts: a part of it useful in certain organisms, or even certain cells, in maintaining a bodily or cellular temperature which is the optimum or so-called "normal"; and another part, in excess of this first part, which is waste, to be gotten rid of as promptly as any other waste. It is a dangerous product, as the organism in fever shows.

We may, then, regard the end products of respiration, the

oxides and the heat, as wastes. Although inevitable they are not essential; some of them, possibly all, are dangerous, and for this reason to be eliminated as quickly as possible.

The bodies of animals are compact masses compared to the bodies of plants. From the extensive surface of the plant body radiation is rapid; from the limited surface of the higher animals, it is slow. The plant needs no forced draft to carry off its waste heat; the massive animal does. The submersed animal is in a medium which quickly absorbs heat; it needs little else than the water it lives in to relieve it of its waste heat, as well as its waste oxides. Its simple circulatory system is adequate. But air absorbs little heat; it is indeed a fair insulator as compared with water, as the bather in cold water knows by experience.

The liberation of heat may be used, like carbon dioxide, by the physiologist as a gauge of the activity of respiration, but like carbon dioxide, it must be regarded by him as an end product, a waste, and not the essential product. The essential product of respiration may be energy, but if so, it is that energy which is immediately convertible, and is converted into work by the organism. On the other hand, respiration may be essentially a process of purification, in which useless or injurious substances are converted into forms which can be eliminated. The liberation of energy accompanies these oxidations. Some of this energy may be useful and used; much of it is useless and is eliminated. Elimination by radiation is sufficient in organisms of extensive area in proportion to their mass. Radiation is insufficient for organisms of small area in proportion to the mass. In these the circulatory and so-called respiratory systems are employed to eliminate heat as well as the material products.

In this study of heat liberation, therefore, I believe I have been occupied with an unessential, although inevitable, feature of the process of respiration. The essential part of the process of respiration is much more likely to be found to be chemical and not physical. And if this is made any more evident by this study, my work is not in vain.

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TYPES OF CUBAN TOBACCO

HEINRICH HASSELBRING

(WITH PLATES IV–X)

One of the most persistent ideas in evolutionary writings which deal with cultivated plants is that transferring a plant from one region to an entirely different one, or from one environment to another, is accompanied by unprecedented variability during the first season of growth after the transfer. The phenomenon is generally described as "breaking up of the type."

When Cuban tobacco seed is grown in the United States, the crop produced is not uniform, but consists of a mixture of many different forms. SHAMEL,[1] who has made extensive experiments with the introduction of Cuban tobacco into the United States, describes this phenomenon and attributes the appearance of a diversity of forms from the imported seed to the variability induced by the new environment. Regarding the variability of plants from seed imported from Cuba and Sumatra, SHAMEL and COBEY[2] say:

> The plants grown from this freshly imported seed broke up into many different types. . . . . This breaking up of type is due to the effect of the change of soil and climatic conditions, resulting in striking variation in the plants grown from the imported seed. The variation is particularly marked where southern seed is taken to northern tobacco districts.

Similar views are expressed in the older literature. Thus LOCK,[3] speaking of the importation of Havana tobaccos into other countries, says:

> There is no great difficulty in raising plants of these varieties, but they speedily degenerate and form new varieties, if the climatic conditions, etc., are not favourable.

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[1] SHAMEL, A. D., The improvement of tobacco by breeding and selection. U.S. Dept. Agr. Yearbook 1904:435–452. *pls.* 7. *figs.* 2.

[2] SHAMEL, A. D., and COBEY, W. W., Varieties of tobacco seed distributed in 1905–1906, with cultural directions. U.S. Dept. Agr., Bur. Plant Ind. Bull. 91. pp. 38. *pls.* 9. 1906.

[3] LOCK, C. G. W., Tobacco: growing, curing, and manufacturing. London. 1886. pp. 32.

Recently similar phenomena have been described by COOK[4] and his collaborators as occurring in cotton when transplanted from one locality to another. It is evident from these citations that it is a common belief that plants, when transported from one environment to another entirely different one, tend to break up into a number of new types. This view is held by many writers, especially regarding tobacco.

During the years 1907 to 1909, while connected with the Cuban Experiment Station at Santiago de las Vegas, I was able to gather a number of facts and to carry out some experiments which lead to a different interpretation of the phenomena observed when Cuban tobacco seed is imported and grown in the United States. The results of these observations and experiments are given in this paper. For a complete understanding of the phenomena in question, three phases of the subject are considered: (1) the composition of so-called Cuban tobacco; (2) the special methods of agriculture which tend to influence or maintain the present composition of Cuban tobacco; and (3) cultural experiments with types of Cuban tobacco.

The composition of Cuban tobacco

Even a casual survey of the tobacco fields of western Cuba shows that the crop in any field lacks entirely that uniformity which is characteristic of any field of plants of one variety in regions of more advanced agriculture. The plants show a great variety of forms, which at first seems bewildering. Upon closer study, however, it is seen that some types predominate. Most of the plants may be divided into groups, the members of which resemble each other more or less. It is impossible, however, to delimit such groups definitely, or to class all the plants into groups by a taxonomic study. Certain types are well marked, but the number of minor forms, differing in width and length of leaves, habit, branching, etc., is so great that the whole appears to be a mixture of innumerable intergrading forms. While, as has been stated, some of

[4] COOK, O. F., Local adjustment of cotton varieties. U.S. Dept. Agr., Bur. Plant Ind. Bull. 159. pp. 75. 1909.

COOK, O. F., MCLACHLAN, A., and MEADE, R. M., A study of diversity in Egyptian cotton. U.S. Dept. Agr., Bur. Plant Ind. Bull. 156. pp. 60. *pls.* 6. 1909.

the forms occur with great frequency and form the predominating elements of the mixture, others are rarer, although distinctly marked. Some of the more striking forms can be recognized and described taxonomically, but for the majority of the intergrading forms cultural work is necessary in order to determine their constancy. The condition here described exists, so far as I have been able to determine by examination of the fields or by growing seeds from different sources, in all the tobacco fields of western Cuba, or the regions known as the Vuelta de Abajo and the Partidos districts. It is not likely that the tobacco fields of the eastern district will be found to differ in composition from those of the other regions. The condition shows that there has been no systematic effort directed toward the amelioration and improvement of the tobacco plant in Cuba. The persistence of the present condition is explained by the special methods of agriculture in vogue in Cuba.

Methods of agriculture

In the regions of Cuba which have been long under cultivation, great difficulty is experienced in growing *posturas*, or young tobacco plants. The soil is so thoroughly infected with fungi that a rain at any time during the season for growing *posturas* is sure to result in the entire destruction of the plants in the seedbeds. I have seen many acres of seedbeds in the finest condition destroyed in a few days by fungi following a heavy rain. As a result of this, it is customary to grow a large part of the *posturas* in the newer lands in the mountains, in soil which is partly sterilized by burning brush on the surface. The *posturas* are tied in bundles, which are packed in large bales and sent to the various tobacco districts of the island. Any grower who has lost his *posturas* makes up the supply by purchase from the mountain growers. Many growers depend entirely on these mountain-grown *posturas*, seed for which is gathered in various parts of the island. Whether the grower is gathering seed for sale or for his own use, no attempt is made to select seed from the best plants. All the plants in the fields are topped and harvested. It is not even customary to allow any of the plants to flower on the main stem and produce seed. After the harvest of the leaves, the stems are cut off close to the ground,

and the field is abandoned, receiving no further irrigation, which is necessary during the growing period of the crop. As a rule, there is sufficient moisture in the soil to produce a crop of suckers from the old roots. These make a weak growth among the weeds of the abandoned fields and produce flowers and seeds. It is this crop of suckers which furnishes the seed supply for the new crop of tobacco.

Under these conditions, any form of selection is impossible, for the sucker shoots do not show the characteristics of the parent plant. When the seed is mature, all forms, good and bad, are indiscriminately gathered and resown the following season.

These methods of obtaining seed and growing *posturas* bring about two results: (1) all types of tobacco that occur in Cuba are maintained there by the blanket method of harvesting seed indiscriminately from all kinds of plants; and (2) by reason of the traffic in *posturas* and seeds, all types are distributed to all the tobacco-growing regions, so that a uniform mixture of types is maintained over the island.

Cultural experiments

As has been stated, a study of the plants in the field is not sufficient to disentangle the mixture of types and lead to exact information regarding their constancy. To determine whether these types are constant, or whether Cuban tobacco possesses the enormous variability usually attributed to it, cultural experiments were begun in 1908. During the tobacco season of that year, about 30 plants were selected which seemed to represent distinct types. A careful description, recording all characteristics that might be of any value in identifying the types, was written for each plant. The plants were staked and labeled and given a number. Inasmuch as the plants had been topped, it was necessary to save seed from the suckers which appear at the base of the plant after the stem is cut. A number of the plants did not form suckers, so that only 14 plants remained. The suckers selected for seed were inclosed in manila bags in the usual way, while the others were cut as soon as they appeared. In this way, guarded seeds from 14 isolated, self-fertilized mother plants were obtained.

In harvesting and separating the seeds from the capsules, of course every precaution was used to avoid mixing the different types. Each bag was taken separately and the seed shelled out in a large porcelain evaporating dish in the laboratory. The different lots were handled in such a way that there was no possibility of a stray seed being blown or scattered among those of another lot.

Similar precautions were used in sowing the seeds the following autumn at the beginning of the next tobacco season. The seeds were sown in flats, in soil taken from a nursery where no tobacco had been grown, and distant from any tobacco field. The soil was sterilized with hot water. After the seeds were sown, the flats were covered with burlap frames, and were protected from ants, which carry off the seeds, by a ridge of powdered naphthalene around the edge of each flat. The posts supporting the benches on which the flats stood were kept wrapped in cloth soaked in crude oil. The benches had been previously freed from ants by boiling water. With these precautions, no trouble was experienced from the insects. The flats were kept in an open shed.

The seeds were sown September 16 and 18, 1908. When the seedlings were large enough, they were pricked out in open frames kept covered for a time with canvas. The *posturas* were planted in the field at various times from November 12 to December 9, 400–500 plants of each type being set out.

The results of the cultures were so striking and uniform that they can be stated in a few words. Even in the open frames the various groups of plants showed differences which made them stand out from each other, but the differences were more evident when the plants were mature. The descendants of each plant were entirely uniform and like the parent plant from which they were derived. Even minute and unimportant characters were transmitted with surprising definiteness.

While the different types were indiscriminately intermingled in the field, the contrast even among extreme types was obscured on account of the many apparently intergrading variations, but in the cultures where large numbers of each type were grouped together the differences were unmistakable. Thus, for instance, the different groups as a whole showed marked differences in height,

a characteristic which was not evident in the field, where owing to individual variation the stature of a plant is not a pronounced characteristic. Yet when the descendants of individual parent plants were brought together in groups under uniform conditions, the contrast in stature between the different groups was constant and very marked.

The more important morphological characteristics in which the various types differed from each other were the shape of the leaves and flowers, and the form of the inflorescence, but the descendants of a single parent were entirely uniform with respect to these characteristics. Even such minor characteristics as the tint of the leaves and color or shade of the flower were uniform throughout the plants of each group. In some cases the same type had been selected more than once, so that some of the groups were identical. The plants in the field were studied during their entire growing season, and each individual was often examined, but among the several thousand plants no aberrant form occurred.

The uniformity of the descendants of each of the parent plants indicates that the plants originally selected represented elementary species, for if the parent plants had been hybrids, splitting according to Mendel's law, the splitting should have occurred in the generation of 1909. The absence of hybrids among the plants selected can be explained by the ease with which self-pollination takes place, and by the scarcity of pollinating insects. The flowers are slightly proterogynous, but even before the flowers are fully open the anthers begin to shed their pollen. As both pistils and stamens are about equal in length, self-pollination is easily accomplished. Pollinating insects seem to be rare. During the two seasons in which I spent much time in the tobacco fields, I observed only in a few instances honey bees and hawkmoths pollinating flowers. It seems very likely, therefore, that in the majority of cases the tobacco flowers in Cuba are self-pollinated.

In order to continue the pure line cultures, a large number of plants of each type were bagged for seed in the usual way, but on account of my removal from Cuba in the spring of 1909, seeds from all the types were not obtained. Some clusters with mature seeds were found on 11 of the types. The seeds from 10–15 plants of

each of these types were collected and brought to the United States. These seeds were grown at Flint, Mich., during the summer of 1910.

It is in the first crop from seed imported from Cuba that the splitting of the type into numerous varieties has often been reported. The plants in the pure line cultures in Michigan, however, showed no signs of such splitting. The plants resulting from the mixed seed of 10–15 plants of each type were entirely uniform and similar to each other. In all their important morphological characteristics they were identical with their parent plants grown in Cuba the year before. In some minor characteristics some types differed from the plants grown in Cuba. The leaves were of a darker green and the plants generally were taller and more vigorous in the more fertile soil of Michigan. In so far as there was any detectable influence due to the new environment, all the plants of a particular type reacted alike.

Discussion

It appears from the foregoing experiments that when pure strains of tobacco are selected from the mixture grown in Cuba and brought into a new environment in the United States, these pure strains show no breaking up of the type due to the new environment. The slight changes which are observed in the plants affect all the plants of one type alike.

The effects observed by SHAMEL and COBEY and others are attributable to the fact that the seed was derived from a mixture of types. Since a great number of types occur in the fields of Cuba, it is not necessary to invoke the doctrine of "breaking up of types" to account for the appearance of numerous varieties when Cuban seed is sown in the United States.

The same principle applies to tobacco and other plants cultivated in countries where agriculture has not reached a high state of development, and where the concept of an agricultural or horticultural variety hardly exists. In Cuba I have cultivated tobaccos from a number of districts in Mexico, and find that these are also mixtures of types which resemble in their general appearance the Cuban types and probably belong to the same group of elementary

species. Recently HOWARD and HOWARD,[5] in their excellent studies on Indian tobaccos, have isolated 51 types from the tobaccos grown in India, and have shown that these types remain constant even in minute and insignificant characteristics when propagated in pure line cultures. SHAMEL[6] also has found and repeatedly emphasized the fact that when seed is obtained from single self-fertilized mother plants, the progeny is entirely uniform. In one instance he reports that plants from Florida-grown Sumatra seed showed great variability for two generations when grown in Connecticut, the seed being collected in the ordinary way from many plants, but when seed was saved from single mother plants in the second generation of northern grown plants the offspring of these plants were uniform. The variability continued for two generations, but when seed was collected from isolated plants the environment had no further effect!

COOK[7] found an exactly analagous behavior in cotton. When seed was saved from individual mother plants selected from the diverse forms of King cotton grown at San Antonio, Tex., the offspring of these forms were either uniform or showed definite types of variation. The occurrence of definite types of variation would seem to indicate that the parents were hybrids.

It is scarcely believable in either the case of tobacco or of cotton that a single selection would destroy the plant's capacity for being affected by its environment. In tobacco variation has been reported to persist at least during two generations in the new environment, yet from individual plants selected at any time a pure progeny was obtained. All such facts are more easily understood on the basis that the seed was derived from mixed parents.

It is true, of course, that plants are modified in their fluctuating characteristics by changes in the environment, but so far as experimental evidence shows, such modifications persist only as long as the environment inducing them persists. LE CLERC and LEAVITT,[8]

[5] HOWARD, A., and HOWARD, G. L. C., Studies in Indian tobaccos. Mem. Dept. Agr. India (Bot. Ser.) **3**:59–176. *pls. 58.* 1910.

[6] SHAMEL, A. D., *loc. cit.*, Yearbook. 1904. [7] COOK, O. F., *loc. cit.*

[8] LE CLERC, J. A., and LEAVITT, S., Tri-local experiments on the influence of environment on the composition of wheat. U.S. Dept. Agr., Bur. Chem. Bull. 128. pp. 18. 1910; rev. in BOT. GAZ. **50**:153. 1910.

in their work with wheat, showed that this influence of the environment is exerted also on the chemical composition of plants. When wheat of one variety from one locality was grown in other localities, with a widely different environment, the chemical composition of the grain was different in each locality. These differences persisted as long as the wheat was grown in the particular locality, but if at any time seed from one locality was grown in any of the others, the grain took on the composition of the wheat constantly grown in those localities. The tobacco plant is extremely susceptible to changes in the environment, but such changes affect all the plants of a pure strain alike, and do not cause a breaking up of the type. Among the plants grown in Michigan, some of the types showed a different shade of green from that shown by the same types in Cuba, but all the plants changed alike.

Description of the types

The taxonomy of the TABACUM section of the genus *Nicotiana* is endlessly confused, and it is not possible from the materials at hand to give a proper classification of the forms involved in these studies. Many of them are undoubtedly well defined species. The facts that they have maintained themselves for a long period of time, that no effort has been made to improve them, and that they resemble races from Mexico, seem to indicate that these forms are not far removed from the original wild species of tobacco. As their definite classification would require the study of vastly more material than is at my disposal, it seems best to indicate the general relationships of the types and give the main characteristics by which they were distinguished. For similar reasons, it is useless to speculate on the origin of the forms of tobacco which now occur in Cuba. Many of the growers have a vague idea that the tobacco of today is not the real Cuban tobacco famous in former times. It is a common belief among them that during the long wars tobacco growing was almost exterminated on the island, and that subsequently tobacco was imported from Mexico, Porto Rico, and other regions. It is more probable, however, that even in early times the tobacco of Cuba consisted of a mixture of forms. As early as

1722, LABAT[9] describes four forms cultivated on the islands of tropical America, and it is quite probable that these were generally distributed over the islands.

In the following pages a brief description and history of the 11 types cultivated both in Cuba and Michigan are given. In designating the types, the same numbers are used by which they were designated in the cultures. In the accompanying plates showing leaves of the different types, each type shown is represented by all the leaves of a single plant of that type.

Broadly speaking, the types may be divided into two groups. The plants of the first group are characterized by broad, rounded leaves, short in comparison with their width, and scarcely or not at all pandurate, but sessile by a broad base and decurrent auricles; or the lower leaves contracted into a somewhat pandurate shank. The base or shank of the leaves in general is not as much wrinkled as in the second group. The upper small leaves on the stem and subtending the branches of the inflorescence are ovate, acute, or barely acuminate. The corolla tube is suddenly inflated just above the middle, and the limb is pentagonal and obscurely or scarcely lobed, the lobes being apiculate. The plants of this group probably constitute the collective species *Nicotiana macrophylla*. The group will therefore be referred to as the *macrophylla* group.

The plants of the second group are marked by oblong-ovate leaves, longer in comparison with their width than those of the first group. They are more pointed, often acuminate, and have the base contracted into a pandurate, very wavy and wrinkled shank, with broad, decurrent auricles. The uppermost leaves are ovate to lanceolate and long-acuminate. The corolla tube is trumpet shaped, gradually expanding from the base, with the stellate limb distinctly and often deeply lobed, with sinuate-acuminate lobes. This group is more heterogeneous than the former, and makes up the greater part of the tobacco grown in Cuba. The typical forms of this group probably constitute the collective species *Nicotiana havanensis*. Some of the less typical forms may belong to others of the older species.

[9] LABAT, JEAN BAPTISTE, Nouveau voyage aux isles de l'Amerique. 4to ed. **2**:166. 1724; also 12mo ed. **4**:476. 1724; the edition of 1722 was not seen. See also Du Tertre Jean Baptiste Histoire générale des Antilles. **2**:99. 1667.

THE MACROPHYLLA GROUP

No. 7: pl. IV. fig. 1; pl. VI. fig. 7; pl. X. fig. 14

No. 7 is a dark green vigorous type which grew to a height of 2 m. in Cuba and 2.5 m. in Michigan. In the field growing near the other plants of the *macrophylla* group, the plants of this type stood out in strong contrast by reason of their darker color and tall growth, and their loose spreading inflorescence. The leaves are rather thick and firm in texture. This type is not liked by Cuban growers, some of whom saw it in the experimental plats.

Nos. 16 and 28: pl. VII. fig. 9

Nos. 16 and 28 proved to be identical. They are a broad-leaved type, differing from no. 7 in their dwarfer habit and more compact growth, as well as in their paler green color. The whole inflorescence is more compact than that of no. 7, and the flowers are paler. When grown side by side, these two types show a uniform and striking contrast with no. 7.

No. 18: pl. IV. fig. 2; pl. VIII. fig. 11; pl. X. fig. 15

No. 18 has the general habit and characteristics of nos. 16 and 28, which it resembles more than it resembles no. 7. This type is distinguished from the others by its remarkably large broad leaves, which are soft and flaccid, so that they appear wilted in the sun. Some plants of this type grown in Cuba during the summer season showed all the characteristics of the parent plants which had been grown during the previous winter. In Michigan the leaves grew to a very large size, but remained soft and flaccid, differing clearly from the more turgid leaves of the other forms.

THE HAVANENSIS GROUP

No. 25: pl. IV. fig. 3; pl. VIII. fig. 10; pl. X. fig. 17

No. 25 has broadly ovate or oblong leaves, arching at first and later deflexed, narrowed into a distinct pandurate, wavy, and bullate shank, which expands again into large wavy auricles terminating in long decurrent wings. The upper leaves are ovate to ovate-lanceolate and acuminate. This form may be taken as

typical of the bulk of the tobacco grown in Cuba. By far the greater part of the plants in the fields belong to this type, and differ from the particular strain here described only in minor details, such as shade of the flowers, length and breadth of the leaves, and height of the plants. These minor variations, however, seem to be transmitted with great fidelity in strains descended from a single plant. Cuban growers who saw the plats uniformly picked out such types as this and nos. 36 and 37 as *Tabaco criollo*, which signifies that it is the pure Cuban. The broad-leaved types, as well as the narrow-leaved forms mentioned farther on, were generally regarded with disfavor by the Cuban tobacco growers. From these facts it seems probable that this type represents the typical Cuban tobacco cultivated in the early history of the Island and known in horticulture as *Nicotiana havanensis*.

Nos. 36 and 37

These two forms are also typical Cuban forms and differ from no. 25 only in the width and length of the leaves. The difference in width of leaves of the different strains, while very slight, was still apparent when the plants stood in groups side by side. Too much stress should not be laid on differences of this nature unless accompanied by other characteristics, or unless the differences can be clearly defined by plotting the variability curves. For all purposes, except those having in view the selection of superior strains, these types can be grouped with no. 25 as typical Cuban or Havana tobacco.

No. 12: pl. V. fig. 4; pl. VII. fig. 8; pl. X. fig. 18

No. 12 is a Cuban type, but differs from the foregoing forms in several ways, so that it was readily distinguished. The leaves were thicker, more rigid, and narrower than the other forms. In Cuba the leaves had a peculiar gray-green color which contrasted sharply with the neighboring plants. In Michigan this difference in color was less conspicuous, but all the plants of this type reacted alike as to the loss of color. The flowers are deep rose. On account of the stiffness of the leaves, their distance on the stem, and the smallness of the upper ones, the plant has an open habit quite distinct from the larger-leaved types.

No. 5: pl. X. fig. 16

No. 5 is a Cuban type with unusually broad leaves, which are obtusish at the apex, and with the shank not so distinct as in the other types. The form tends toward the broad-leaved types. The flowers are white or very pale pink, with the limb of the corolla lobed as in the other Cuban types. The plants retained all their characteristics in Michigan.

No. 32: pl. V. fig. 5; pl. IX. fig. 12

No. 32 is striking on account of its strict habit and peculiar deep bluish-green color. The leaves are of the narrow type, oblong to oblong-ovate, erect, forming an acute angle with the stem, and with the apex long-pointed and arching. The surface is marked with furrows and the pandurate base is much wrinkled. The flowers are deep pink to pale pink, with triangularly lobed limb. The strict habit and thick leaves of dark bluish-green color make these plants very conspicuous in the field. The characteristics of the plants were retained in Michigan.

No. 1: pl. V. fig. 6; pl. IX. fig. 13; pl. X. fig. 19

All through the Cuban tobacco fields there occur narrow-leaved forms which resemble the ordinary Cuban types to some extent, at least while they stand intermingled with them. It is all the more difficult to separate these forms definitely because a number of gradations occur from very narrow leaves to much wider-leaved forms approaching the typical Cuban tobacco. These forms do not constitute a large percentage of the tobacco crop. They are sufficiently conspicuous, however, to have attracted the attention of Cuban growers, who call such forms *lengua de vaca* or cow's tongue. A number of such forms varying in width of leaves were selected, but seeds were obtained from only one form. The others failed to produce suckers. No. 1 is a conspicuously narrow-leaved form. The plants are of dwarf habit, having the large leaves low down on the stem, thus giving the upper part a naked appearance. The leaves vary from oblong-pointed to lanceolate-acuminate. The surface is wavy, being obliquely furrowed parallel to the veins. The base is narrow-pandurate with decurrent

auricles. Inflorescence loose, open. Limb of the corolla deeply lobed, with ovate or triangularly acuminate lobes. Color pale rose to deep rose. In Michigan the form retained the same dwarf habit, with the large leaves on the lower part of the stem. The only difference was that the plants grew more vigorously and had larger leaves in the northern habitat. Types like this and no. 32 differ very conspicuously from the other members of the *havanensis* group, and it is possible that they belong to other species.

Conclusion

The tobacco grown in Cuba consists of a mixture of a large number of forms which maintain their characters from generation to generation. Pure strains, breeding true to type, can readily be selected from this mixture. When such pure strains are grown in northern climates, they do not break up into a number of new types, but the plants of each strain remain uniform. Such modifications as appear, appear alike in all the plants of a given strain.

BUREAU OF PLANT INDUSTRY
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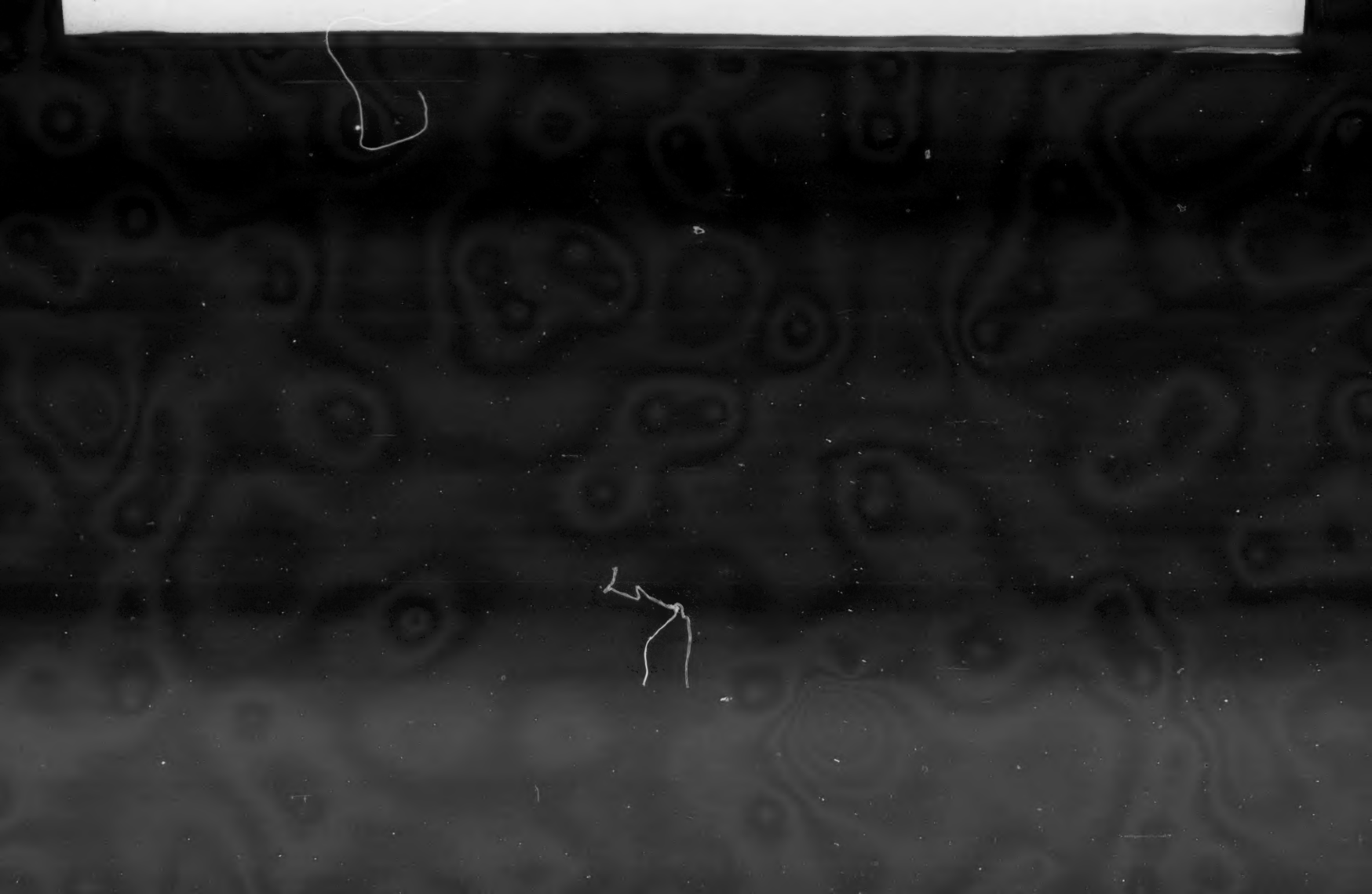
HASSELBRING on CUBAN TOBACCO



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HASSELBRING on CUBAN TOBACCO

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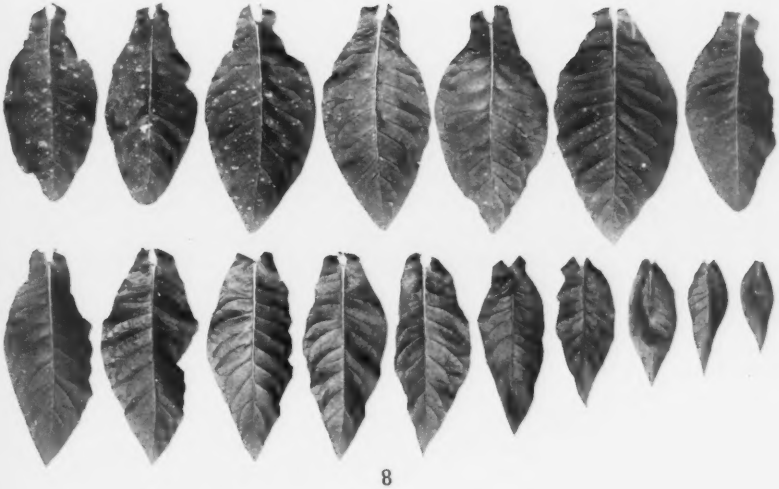

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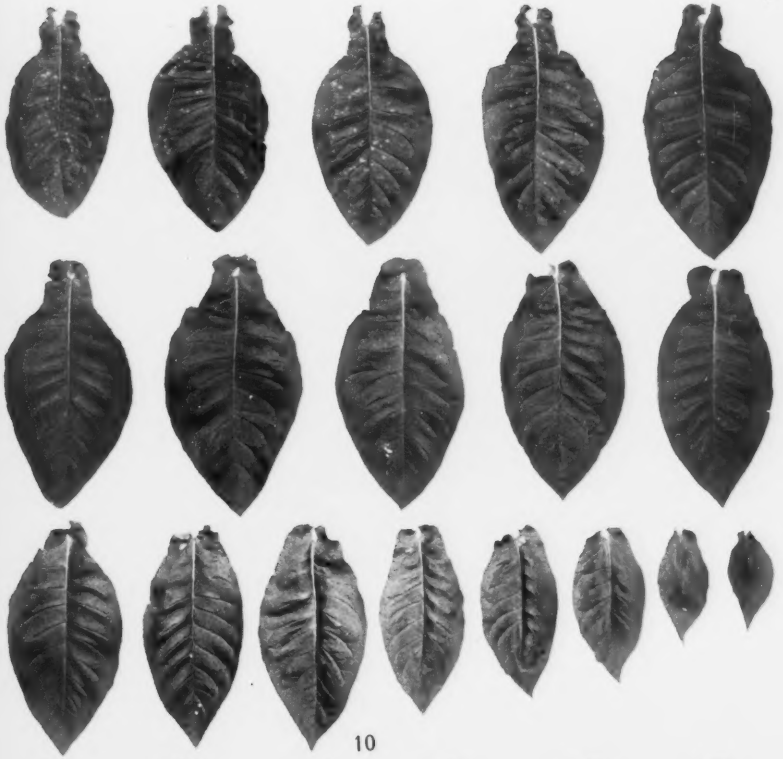

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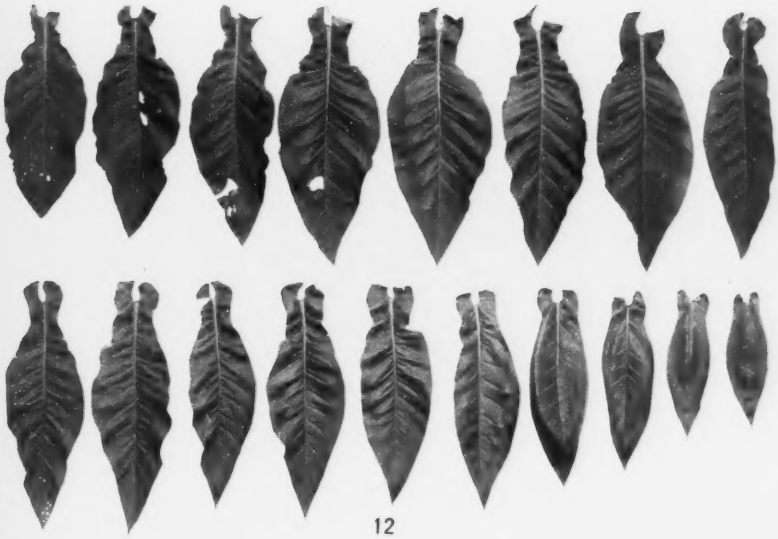

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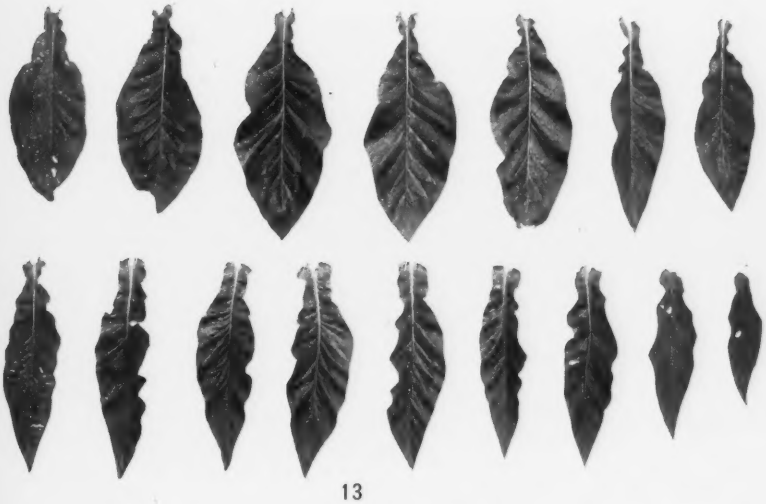

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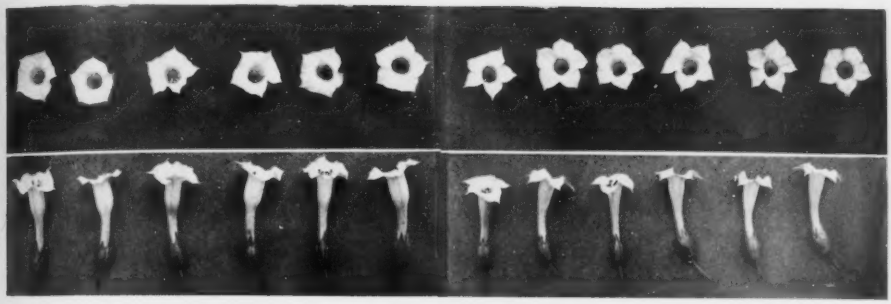

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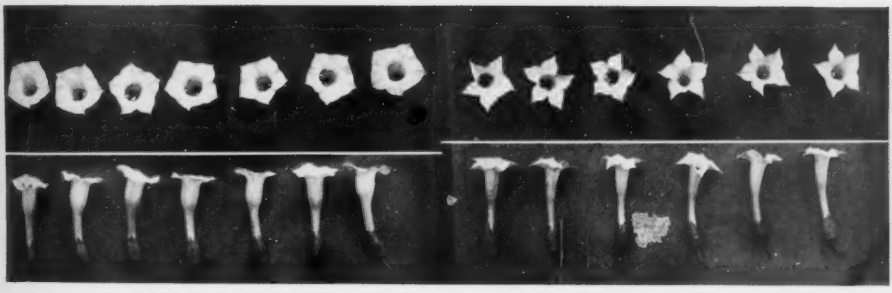

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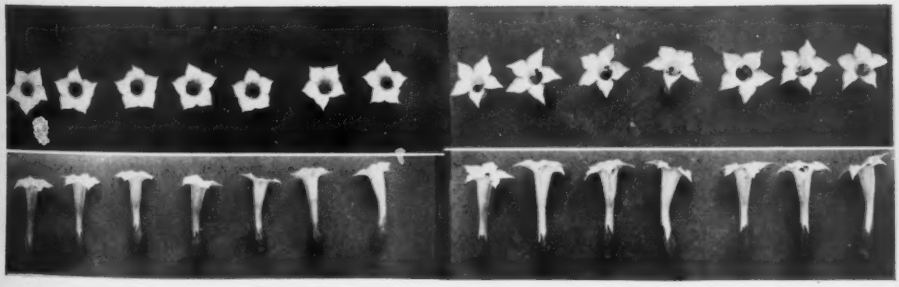

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HASSELBRING on CUBAN TOBACCO

THE DEVELOPMENT AND CYTOLOGY OF RHODOCHYTRIUM[1]

ROBERT F. GRIGGS

(WITH PLATES XI–XVI)

To the student of phylogeny, whether he be taxonomist, or morphologist, no organisms are as interesting as those which appear to occupy positions intermediate between the larger groups and help to fill the gaps in our evolutionary system. Such a form is *Rhodochytrium*, for it seems to occupy a transitional position between the protococcoid algae and some of the chytridiaceous fungi. It was described by its discoverer as an alga, but it has no chlorophyll and is strictly parasitic in its mode of life, being limited, moreover, to definite host species. Although entirely incapable of photosynthesis, it develops abundant starch. But the starch grains are apparently built up directly in the cytoplasm, for neither plastids nor pyrenoids have been found. This paradoxical combination of characters aroused in the writer a desire to investigate the details of its structure and to compare its cytology with that of *Synchytrium*, which has proved so peculiar.

As is well known, *Rhodochytrium* has been found only in three widely separated regions. LAGERHEIM observed it in many places in Ecuador on *Spilanthis* sp., and his material has been distributed in WITTROCK and NORDSTEDT'S *Algae Exsiccatae* as no. 1096. BARTHOLOMEW discovered it on *Asclepias pumila* about 20 miles from Stockton, Kansas, and distributed it as *Fungi Columbiani* no. 2166 (forma *asclepiadis* Farlow); and finally STEVENS and HALL[2] have found it on *Ambrosia artemisiifolia* in many places in North Carolina, as reported by ATKINSON (3). BARTHOLOMEW has informed me that the plant is rare in Kansas and known to him from only one locality, but both in Ecuador and North Carolina

[1] Contribution from the Cryptogamic Laboratory of Harvard University, no. LXV.

[2] MR. HALL has also found it at Clemson, South Carolina.

it is widely distributed and common. It is in each case, however, except possibly in Kansas, closely limited to the particular host on which it was reported.

The parasite attacks all the aerial parts of its host, but, like certain species of *Synchytrium*, it is largely confined to the tissues immediately adjoining the vascular bundles. To the naked eye each parasite appears as a small bright red spot buried in the tissue of the host. When a piece of the infected tissue is examined under the microscope, it may be seen that the parasites are of two sorts, resting spores and zoosporangia. The resting spores are somewhat deeply buried in the tissue of the host, but their superficial origin may be demonstrated by the persistence of the original germ tube with its external button, *Cystenhäut* (fig. 4), through which the parasite penetrated the host. The zoosporangia (fig. 28) are irregularly turbinate or retort-shaped bodies with wide flaring necks, through which their contents are emptied at maturity as biciliate zoospores which spread the infection during the growing season. From the basal portions of both sorts of cysts numerous rhizoids are given off, which penetrate the vascular bundles of the host, especially their phloem elements, and gather nutrient for the parasite.

In carrying out the investigation I have been aided to an unusual degree by my friends. I desire to extend my thanks and acknowledgments to Messrs. F. L. STEVENS and J. G. HALL of the North Carolina Experiment Station for the material, especially to the latter gentleman, who has put himself to no little inconvenience in killing material at all hours of the night as well as in seasons of the year when it was difficult to secure; to Professor GEORGE F. ATKINSON, who himself planned to make detailed studies upon the plant, for the generous way in which he encouraged me to proceed with the present investigation; and to Professors ROLAND THAXTER and W. G. FARLOW for the courtesies of their laboratory and for much valuable criticism and advice.

The material was killed at Raleigh in chromacetic acid and shipped to Columbus in the killing fluid, after which it was dehydrated and imbedded in paraffine in the usual way. The safranin-violet combination proved most satisfactory as a stain. Iron

haematoxylon, for some reason, was hard to handle with this material.

Observations on living material

Through the kindness of Mr. HALL, supplies of infected ragweed were sent to me at frequent intervals. By this means it was possible to determine the approximate sequence of events through the year. It would be interesting to compare the seasonal cycle of the parasites in North Carolina and in Ecuador, but LAGERHEIM has left us no data concerning the seasonal history of his plant. In regard to the characters of the living cysts and the behavior of the zoospores, I cannot add in any important particular to LAGERHEIM'S account, though my observations confirm his at almost every point.

According to my observation, the parasite does not appear at Raleigh until rather late in the season. Seedling ragweeds, gathered among the stubble containing the old resting spores on April 20 and May 20, showed no infection on arrival in Columbus, and did not subsequently develop any when grown in the greenhouse. Young plants gathered May 31, however, showed a few parasites. At first nearly all of the cysts become zoosporangia, but before June has passed, resting spores begin to appear in numbers, the zoosporangia become gradually scarcer and scarcer, until finally, about August 1, practically the only cysts found are the quiescent resting spores which undergo no further change until the following spring. These are even more conspicuous than the zoosporangia, but for any observations, either biological or cytological, material must be gathered while zoosporangia are still abundant, that is to say before the middle of July, preferably during the latter half of June.

With a little care the two sorts of cysts can be distinguished in the living state under a hand lens. The resting spores are more regular in shape and more deeply buried than the zoosporangia, and they are usually more deeply pigmented, since their protoplasm is more compact and less vacuolate.

The first infections observed were mostly on early leaves, which soon wither and drop off in the natural development of the plant,

whether parasitized or not. But before these leaves wither, the parasites they contain ripen and discharge their zoospores, which carry the infection to the younger parts nearer the growing point of the host. In this manner infection is carried to successively higher and higher levels of the growing plant, until the host is often red with parasites.

LAGERHEIM believed that the cysts arose not only by direct infection but also by proliferation from the mycelium of old ones. If this occurred, a single infection might, by repeated proliferation, infect every part of the host plant. But in the form on *Ambrosia* no indication of such proliferation was found. Nowhere among the rhizoids were any indications observed of the formation of new growing points or other signs of proliferation. During the whole of the growth period the parasite is strictly unicellular, with a single nucleus in the body of the cyst, and when nuclear division begins preparatory to sporulation, the nuclei do not wander into the rhizoids. In sectioned material only a small proportion of the parasites are so oriented that a single section passes centrally through the whole cyst. But in no case, where the series was complete, was there any difficulty in finding the external opening of any zoosporangium, whereas if proliferation had been occurring, numerous partially formed cysts which had not yet grown out to the epidermis should have been encountered. In those parasites which become resting spores the independence of the cysts cannot be demonstrated by finding their external openings, because, on account of the narrowness of their necks, only a small proportion of them can be followed to the exterior. If proliferation occurred, the new cysts could become nucleated only by migration of nuclei through the rhizoids. But not only do the nuclei of the resting spores remain undivided, but they have not been seen to wander from their central position in the middle of the cyst, and they are so large that it is difficult to imagine them squeezing through the rhizoids.

Although the parasites are so abundant as almost to cover the host plant, and the rhizoids destroy the cells which they penetrate, the vigor of the plant is little impaired. But when infected ragweeds are transplanted, it is difficult to prevent the parasitized

leaves from withering and dying, and reinfections on healthy portions of the plant are difficult to secure.

The most convenient way to obtain the zoospores is to tease to pieces fragments of tissue containing the cysts, liberating the zoospores by rupturing the sporangia. It is difficult to observe the normal exit of the zoospores on account of their minuteness as compared with the massive tissues from which they emerge. But with patience one can study the discharge. LAGERHEIM states that the plug of the zoosporangium is dissolved before the escape of the spores. In only one case was I able to observe the discharge under satisfactory conditions, and then I saw neither the fate of the plug nor the very beginning of the discharge. The whole mass of zoospores appeared to expand as swarming began, and those nearest the opening were forced out in a solid stream by the pressure of those below them. In the case observed they continued to escape at the rate of about 150 per minute for 10 minutes (that is, approximately 1500 spores). The last ones from the rhizoidal end of the sporangium were not at first so well formed as the others, and did not escape with them, but after an interval of 5 minutes began to swarm violently inside the sporangium and some of them escaped one by one. Not all of them were able to find the opening, however, and those which failed became quiescent after about 15 minutes.

Along with the ripe zoosporangia many immature ones, of course, are torn open in teasing apart the material for mounting. Such of these as have advanced far, though not yet mature, are apparently able to form zoospores under the stimulus of rupture. When first discharged the contents of these cysts undergo euglenoid contortions, but in a few minutes become ciliated and break up into spores. Such zoospores, however, are very irregular in size, and abnormal forms compounded of several individual spores are common. Among these are some which might easily be confused with conjugating gametes, being associated in pairs side by side. More commonly such double zoospores are joined at their posterior ends, forming much elongated bodies, pigmented and ciliate at both ends. Frequently a third member is attached to the middle of such a couple, forming a projection at right angles. Others are

large multiple bodies with four or more pigmented areas and many cilia. Such abnormal spores, of course, have very erratic and peculiar movements. Their period of activity is short, few continuing to swim actively for more than half an hour. LAGERHEIM observed these same abnormal spores, and inferred from them that segmentation was successive rather than simultaneous, but, as will be seen, this is not the case.

The zoospores are transparent, except at the anterior end, which is occupied by a mass of pigment. After they come to rest the nucleus can be seen distinctly as a clear central vacuole. In the posterior portions are numerous granules, usually including some starch grains. When moving most actively, the zoospores are oblong rather than pyriform, as figured by LAGERHEIM. Indeed, they appear to be narrower in the region of the nucleus than in the anterior pigmented end. But this is believed to be due to an optical illusion, the more conspicuous region irresistibly appearing larger. It is of course not susceptible of careful observation, since the shape changes at once when they come to rest.

If plentifully supplied with fresh water, the zoospores continue to swim about actively for several hours. In numerous instances they were watched for half a day at a time, and in one case the last one on the slide did not perish until 8 hours after liberation. In preparations supplied with abundant water conjugation occurs but seldom, according to my experience. But when the water has evaporated to a considerable extent, all begin to conjugate at once. When more water was added, those pairs in which fusion had not proceeded too far dissociated rapidly and swam about singly as before. From this it was suspected that conjugation might be due to the increasing osmotic pressure of the medium consequent upon evaporation. On this supposition a few crystals of sugar were added to a similar preparation, making the concentration very much higher than on evaporation, but this had no apparent effect on the zoospores. It was therefore concluded that conjugation was induced by an insufficiency in the quantity of fluid present, and this conclusion seemed to be confirmed when on placing two portions of a culture of zoospores, one in very scanty and the other in abundant water, the first quickly conjugated while the second

did not. The process of conjugation is not different from that common in various algae. Two zoospores of approximately equal size approach (fig. 38) and lie alongside each other (figs. 39, 40); the plasma membranes separating them disappear, and within a few minutes the nuclei, which may be seen as clear central bodies, have fused into one (fig. 41). The two pairs of cilia remain distinct, and in the cases observed by me there persists a slight groove in the anterior portion of the zygote indicating the line of fusion.

LAGERHEIM reports that both conjugated and unconjugated zoospores are able to infect the host. My own observations on this point gave no results. Repeated efforts were made to observe the process of infection, but for the most part the spores swam indifferently about the pieces of fresh ragweed which were placed on the slide with them. In some cases, indeed, the spores, both conjugated and single, settled down on such pieces of the host and became fastened to them with one or more cilia, but in no case did penetration occur.

My attempts at reinfection on the living plant were similarly unsatisfactory. Out of numerous attempts, only three successful infections were secured. In these cases the development of the young parasites was very rapid, but as the successful experiments were my first attempts in that direction, and as all efforts to repeat them failed, I do not feel warranted in reporting them in detail.

One of the interesting questions which the failure of the infection experiments left unsolved is how the character of the young cysts is determined, that is, whether they are to develop into resting spores or into zoosporangia. Although this is connected with the seasonal cycle in North Carolina, there is no indication in LAGERHEIM's account that such is the case in Ecuador. By analogy with other forms, one might suspect that the zoosporangia spring from unconjugated zoospores and the resting spores from zoozygospores. But there is no definite alternation of generations, as in some such forms. In any case, the character of the cyst appears to be determined immediately on infection. As may be seen from the figures, the methods of penetration and growth are different from the very beginning, so that in the very youngest cysts there is no question whatever which are zoosporangia and which are resting spores.

In very few cases was there any ambiguity in this respect, although several malformed zoosporangia were seen. In one of these a heavy wall had formed across the neck, leaving only a small pore between the neck and the body of the cyst. Several very narrow-necked zoosporangia were also observed, but though these resembled the resting cysts in shape, they were apparently otherwise normal.

MICROCHEMICAL REACTIONS.—The two outer walls of the resting spores are cellulose, as reported by LAGERHEIM, who used chlor-zinc-iodide as a test reagent. With iodine and sulphuric acid also they give the cellulose reaction, but were not in my tests as deep a blue as the cotton fibers which were used as a check. But the endospore is different in character and was unaffected by any of the reagents or stains employed.

LAGERHEIM suspected that there might be chlorophyll in some stage of the life cycle, though he was not able to detect it. The plant has more or less red pigment at all stages, but none of my observations gave any ground for supposing chlorophyll to be present.

The red pigment, as reported by LAGERHEIM, is haematochrome or some closely related lipochrome. It is colored green with iodine in potassium iodide, blue with sulphuric and nitric acids, fading away after treatment with the latter. Tests with red individuals of *Sphaerella* under the same cover-glass with *Rhodochytrium* gave somewhat contradictory results, but showed some differences between the pigments of the two. The haematochrome of *Sphaerella* was not dissolved by carbon disulphide, which is a solvent for the allied pigment carotin, even after prolonged treatment, but the pigment of *Rhodochytrium* was easily dissolved under the same conditions. The haematochrome reacted to a weak solution of iodine such as is used for testing starch, but the pigment of *Rhodochytrium* remained unchanged until a strong solution of iodine was applied, when the characteristic reaction appeared. With sulphuric acid also *Sphaerella* reacted instantly, but drops of the red oil of *Rhodochytrium* remained unchanged for several minutes and slowly turned blue. While still inclosed in the unbroken spore, the pigment is very resistant to almost all reagents. This was first noticed on fixing with chromacetic acid, which fades out

almost everything put into it. Nor did the color fade during the prolonged soaking in alcohol and hot chloroform incident to imbedding in paraffine. But when after sectioning it was treated with turpentine, it quickly dissolved. Although easily soluble in carbon disulphide after the spores are broken open, as stated above, it resists that reagent indefinitely (three months) when bits of the host plant containing the spores are treated with it. It was likewise unaffected by three months' treatment with xylol, benzene, chloroform, absolute alcohol, ether, and turpentine. It was also undimmed in brilliance after 6 days' maceration in 10 per cent hydrofluoric acid.

One of the most conspicuous features of the material was the great difference in certain respects between that collected in 1908 and that in 1910. In the former the zoospores (figs. 32–34), and for the most part the zoosporangia also, after the first division (fig. 27) were entirely destitute of starch, their cytoplasm being clear and finely granular. But in the latter the zoosporangia (figs. 15, 27) and almost all of the zoospores as well (fig. 35) were abundantly supplied with starch, which on account of its refractive and staining properties greatly interfered with the observation of nuclear phenomena. The condition of the zoospores was of course reflected in the young cysts, which in 1908 had at first clear granular cytoplasm without a sign of starch grains or any other structures (figs. 2, 3, 14), while in 1910 the cytoplasm was packed with small starch grains from the first (figs. 1, 11, 12). There were also some differences in the nuclear behavior in the two cases. Those figures which are interpreted as amitosis are almost entirely confined to the 1908 material. The plugs of the zoosporangia are also very different in the material of the two years as described below (p. 136). Moreover, there is reason to believe that similar variations occur in the Ecuadorean form, because there are discrepancies between LAGERHEIM'S account and that portion of his material which I have examined, which would be inexplicable to me if I knew only the 1908 material of the form on *Ambrosia*. These differences serve to emphasize the caution we must use in interpreting cytological results. They can hardly fail to suggest that some of the numerous instances where one investigator does not

find what another has reported in a given species, may be due to variations in the conditions of the environment at the time of collection, the effects of which are almost entirely unknown at the present time. From the very character of the work, such errors are peculiarly liable in cytological investigations, for it is manifestly impossible within reasonable limits of time to examine thoroughly material taken under various conditions of growth over a series of years.

The question of species

On account of the great distances separating the three known habitats of *Rhodochytrium* and the diversity of its hosts, one is led to suspect that there are three species rather than one. With the idea of separating them if possible, a study was made of LAGERHEIM'S and of BARTHOLOMEW'S collections. Previous comparison of the North Carolina material with LAGERHEIM'S description had disclosed some minor differences, but these disappeared on examination of the plant itself. In the form on *Asclepias*, likewise, I am entirely unable to detect any constant or significant differences. The various figures presented herewith show how difficult it is to find characters in *Rhodochytrium*. In size and shape there is every possible variation, and there is a total absence of such peculiarities as markings on the spores, etc., which in many groups supply useful specific characters.

It was thought for a time that the shape and size of the plugs which close the mouths of the zoosporangia were different in the three forms. LAGERHEIM describes the original *R. spilanthidis* as having a bell-shaped plug (cf. fig. 21) which did not develop until the sporangium had reached a considerable size. In the form on *Ambrosia* the plug is generally 25–35 μ long, solid, and develops early (fig. 14). The form on *Asclepias* has a similar plug, but it is usually larger, reaching a length of 60 μ (fig. 16). The condition of all the plugs in the 1908 material was fairly constant, but the 1910 material showed such variation that it became evident that the characters of the plug were worthless. Its size varies with that of the sporangium. In large sporangia on the stem it sometimes reaches 50 μ in length, and in small ones on the leaves it is

sometimes as small as 12 μ. Moreover, it is sometimes very tardy in its development. The variation in shape is likewise great (figs. 15, 17, 18, 20, 21, 24). LAGERHEIM'S material shows for the most part the same sort of solid plugs. The form shown in fig. 19 was observed but twice, while bell-shaped plugs such as he figures were entirely absent from that portion of his material which I examined. It seems safe to assume that the apparent discrepancy is to be explained by the same sort of variation as that just noted in the form on *Ambrosia*.

There seems, therefore, to be no course open but to conclude that there is no morphological basis for separating the three forms. There is, on the other hand, reason to expect that they would be found to be physiologically differentiated in respect to their hosts if a series of experiments in cross infection were undertaken with one or all of the forms. Until the experimenter acquires more skill, however, than is possessed by the writer in transferring the infection from plant to plant, the expected negative results of cross-infection would prove nothing.

The range of the plant seems to call for some comment, but the data are hardly sufficient to decide whether the three known localities represent points in a single extensive range, or whether they are isolated stations. If they represent the continuous range of a single species, the limitation to such unrelated hosts raises some considerable difficulties concerning their distribution. Two of the hosts, *Spilanthes lundi* and *Asclepias pumila*, are somewhat localized species, and their range in neither case extends to either of the other stations; but *Ambrosia artemisiifolia* is widespread, and occurs both in Kansas and throughout South America. If the three forms are not physiologically distinct, therefore, cross-infection should occur naturally in Ecuador.

It seems, therefore, that the answer to the question of the number of species of *Rhodochytrium* will depend on the point of view of the student. He to whom geographical and physiological isolation are criteria of species may well conclude that there are three species, while he who demands morphological characters by which to distinguish species will decide that there is but one. Each of these points of view has its advantages, and it is not for

the writer to determine which shall be adopted by his readers. In some groups, as in the bacteria, species are perforce determined almost exclusively by physiological characters, while in other groups, as in the seed plants, morphology alone determines the matter. In the parasitic fungi various infection experiments have shown that numerous species which occur on several hosts may be composed of physiological races, each confined to its particular host. Such a treatment seems to the writer an entirely satisfactory manner of expressing the facts, and he does not see that there would be any gain in considering the forms specifically distinct.

The development of the resting spores

Although the resting spores do not appear in numbers until several generations of zoosporangia have matured and discharged, it will be more convenient to describe them before the more complex development of the zoosporangia is taken up. The very youngest resting spores seen measure about 70 μ in length (fig. 1). They consist of an elongated germ tube with an external button marking the position and size of the zoospore from which they originated. The distal end has already begun to enlarge, but the nuclei (5 μ) are not much larger than those of the zoospores. The germ tubes do not seek out the stomata even when close beside them (fig. 11), but force their way between the epidermal cells at any point. After penetrating a variable distance, usually until a vascular bundle has been reached, the tube begins to swell up and gradually it acquires a globular form. The swelling out of the cyst is very much more rapid than the growth of the protoplast, which in consequence becomes highly vacuolate (fig. 2), like an old cell far back from the growing point in an ordinary plant. There is an attenuate peripheral layer of cytoplasm connected by radial strands with the central body surrounding the nucleus, which likewise has grown but little. At the very beginning of the enlargement of the basal portion, the protoplast withdraws from the narrow neck of the germ tube, which is later cut off by a wall.

Even when full sized, the parasite distorts the tissues of the host but very little. Most of the cells which lie adjacent to it appear as though cut off to make room for its growth rather than crowded

aside by gradual pressure (fig. 15). Generally the walls of these cells can be readily distinguished from that of the cyst, though they may be closely appressed to it. Such walls usually correspond approximately in length with the adjacent part of the parasite. This indicates, especially in those cells that have been much reduced in size, that they have shrunken considerably, for the original wall would have been much crumpled if merely pushed back by the expanding parasite. They often lose their sharp outlines and appear to be undergoing digestion.

The supply of nutriment which makes possible the growth of the parasite is drawn from an extensive system of haustorial rhizoids, which are put out from the basal portion of the young parasite even before the germ tube begins to swell out into the spherical cyst. They continue to increase and to extend their ramifications until the cyst reaches its full size and begins to ripen, finally extending considerable distances along the vascular bundles. But notwithstanding the wide extension of these elements and their filamentous form, they can hardly be compared with the hyphae of a true fungus. They are by no means to be looked upon as the vegetative portion of the plant from which the fruiting bodies take their origin, but merely as rhizoidal outgrowths from the main body of the parasite. When old they develop thick walls, especially in the portions close to the cyst. But at the extremities, where most of the absorption may be supposed to occur, the wall is exceedingly delicate or invisible. Although they sometimes work their way between the disorganizing cells, their course is for the most part within the cells which they invade (fig. 5), and their shape is often largely determined by the boundaries of these cells. Both LAGERHEIM and ATKINSON speak only of those haustorial branches which become attached to the vessels of the system. But the great mass of the rhizoidal system is located in the phloem (figs. 4, 5, 15, 22), and it is the cells of the phloem which are most injured, finally breaking down completely, while the xylem is but little injured. It must also be obvious that the vessels could not furnish the supply of organic food necessary to nourish the parasite. There is no doubt, however, but that some of the ultimate branches of the haustoria do come into close relation

with the vessels, exactly as described by LAGERHEIM and ATKINSON (fig. 5), and probably draw water from them. These terminal haustoria (fig. 6) are closely appressed to the thin places between the spiral thickenings of the vessels, but appear not to penetrate them as in the phloem.

With the development of the rhizoids the protoplasmic contents of the cysts become more abundant and denser. The nucleus increases in size and undergoes a metamorphosis like that of the zoosporangium described below. Starch grains, if not already present, appear and become large and abundant, until they pack the cyst so full that its cytoplasmic contents proper may become almost invisible. In this process all vacuoles disappear and apparently all surplus water is eliminated. Even the aqueous karyolymph partially disappears, causing the nucleus to collapse (figs. 7, 8). In this condition the nucleus differs so far from ordinary healthy nuclei that it is difficult to believe that this change is not pathological. But it seems to be a universal and perfectly normal phenomenon. On the beginning of germination in the spring, the nuclei again become turgid, though they are apparently smaller than before shriveling up.

When the vegetative activity of the parasite is ended, as indicated by the shriveling of the nucleus and the withdrawal of all of the starch from the rhizoids into the spore, a second cellulose wall is laid down on the inside of the spore (fig. 7) and sometimes in the proximal ends of the rhizoids as well (fig. 5). But either at the time of deposition of the second layer of the spore wall or soon afterward, the rhizoids are cut off from the spore first by a plasma membrane and later by a definite wall. This is soon followed by the disorganization of the contents of the rhizoids. The second wall of the spore is quickly followed by the formation of a third (fig. 8), a thick, non-cellulose endospore, which completes the preparation of the spore for its period of rest.

The starch grains

One of the most interesting things about *Rhodochytrium* is the fact that though it is a parasite and has completely lost its chlorophyll, it forms starch in considerable quantities. The source of

this starch is of course the photosynthetic activity of the host, but it is hardly necessary to state that the starch grains of *Rhodochytrium* are quite different in form from those of the adjacent host cells.

As would be expected, starch is most abundant and best developed in the mature resting spores, in which it forms the bulk of the reserve food, but it may be present at any stage in the life cycle. In the zoosporangia it is nearly always present toward the end of the vegetative period, but there is a decided tendency to consume it during the period of nuclear division. A marked difference was noted in respect to starch content between zoosporangia gathered in 1910 and those gathered in 1908. In the former both zoospores and very young sporangia contain numerous starch grains, but in the latter starch appears tardily and almost always disappears before segmentation, leaving the cytoplasm clear and granular, without inclusions of any sort.

The grains seldom exceed 10 μ in diameter and are commonly somewhat smaller. They are usually spherical or somewhat elongated, but very long or double grains are not rare (fig. 9). The larger grains when mounted in balsam frequently show conspicuous cracks at the hilum, as is not unusual in starch grains generally. No definite alternating concentric layers of different refractive indices such as characterize many starch grains could be made out, but in certain grains faint concentric striae appeared to be present. When subjected to the action of strong chromic acid, they show during dissolution the radial structure characteristic of starch grains in general.

STARCH GRAINS UNDER POLARIZED LIGHT.—In the dark field obtained by crossing Nicol prisms, the starch grains show the usual luminous body crossed by dark bars in the two planes of polarization (fig. 10). But there is considerable variation in the behavior of different grains, both in those of the same cyst and in different cysts taken as a whole. Almost all conditions, however, may usually be found in a single cyst. Many of the spherical grains show no change other than the revolution of the crosses when the prisms are rotated, demonstrating in these grains a perfectly symmetrical structure, with the hilum occupying a point in the

center. In elongated grains the crosses resemble those in leguminous starch, namely, a pair of Ys arranged bottom to bottom, indicating an elongated hilum. And in double grains, which are not infrequent, the stems of the Ys are sometimes divided, so that the very center of the grain appears bright. Such grains are of course unsymmetrical, and show the characteristic crosses only when the planes of polarization form the proper angle with the axes of the grain. There are also great differences in the brilliance of the grains; some are very beautiful objects, but others repolarize the light to such a slight extent that they are very faint and the dark crosses are difficult to see. Frequently, indeed, the grains become entirely black and vanish completely when the prisms are crossed. When this happened, I was inclined to suspect that I might have mistaken grains of some other substance for starch, but on running iodine under the cover the characteristic blue reaction promptly appeared to dispel all such doubts.

ABSENCE OF PLASTIDS.—It is unsafe to assert, perhaps, that there are no plastids in *Rhodochytrium*, but it is certain that methods which bring them out clearly in such objects as old potato tubers failed to reveal them in *Rhodochytrium*. So far as could be determined, the starch grains are formed directly in the cytoplasm without the intervention of plastids, pyrenoids, or other specialized protoplasmic bodies. There was only one feature which could be taken to give any indication of such bodies. Many of the grains do not stain uniformly throughout, but show a more deeply colored margin. This appearance is not confined to grains of any particular size, but is found from the smallest to the largest grains. Indeed, when present at all the border is usually wider and more conspicuous in the large grains than in the small. It occurs rather on certain slides or perhaps on certain pieces of material, being present in nearly all of the cysts of some slides while absent from others. The border appears to have the same crystalline structure as the rest of the grain, and seems definitely to be a part of it rather than a separate surrounding body. In no case did it present the granular appearance to be expected of a plastid. I have no satisfactory explanation to offer for this phenomenon, but I do not believe it is permissible to interpret it as a plastid.

Almost ideal conditions for observation of the process of starch formation are sometimes presented in very young zoosporangia (fig. 11), where the cysts are highly vacuolate, with delicate strands of cytoplasm stretched from side to side. In thin sections such strands are suspended across the cyst, with no adjacent objects to interfere with vision. Frequently these strands show all stages in starch formation (fig. 11*a*) from good sized grains down. The larger grains are clear cut, sharply outlined against the clear cytoplasm in which they are suspended. From such well-formed grains there is an unbroken series of smaller and smaller grains down to the limit of visibility. The very earliest stages appear as mere knots in the cytoplasm, while the definite characters of starch grains appear as soon as the body reaches a size large enough to be resolvable into an area rather than a point. At no stage was anything seen in association with the starch grains except morphologically undifferentiated cytoplasm. More often, of course, the grains are formed in large masses of cytoplasm where the opportunities of vision are not so good, but here also they appear to lie naked in the cytoplasm.

The classic examples of the formation of starch grains without differentiated plastids were described by STRASBURGER (**26,** pp. 155 ff.). He found that in the megaspores of *Marsilea* and in the medullary rays of *Pinus* the growing grain was invested by numerous microsomes, which he believed secreted the starch in a manner analogous to the formation of the cell wall by the granules of the spindle fibers at the close of mitosis. These microsomes were large enough to appear as definite granules under a comparatively slight magnification (450 diameters). In *Rhodochytrium*, however, no such microsomes could be made out under a magnification seven times as great.

It should be added also that in those stages where starch is absent the cytoplasm is smooth and granular, without inclusions of any sort. If perchance the writer had overlooked the plastids among the grains during starch formation, he would have expected to see them here, if present. If there are any plastids, therefore, they would appear to be formed *de novo* rather than carried over from generation to generation as permanent organs of the cell.

The development of the zoosporangia

As already stated, the zoosporangia are distinct from the resting spores from the very beginning. The youngest stages seen were approximately as large as the youngest resting cysts, namely, 60–80 μ in length. These future zoosporangia do not form external buttons, and the neck, even at the very first, is of comparatively large diameter (fig. 12). While still very young, the cyst begins to swell out from the initial tubular form, and soon assumes the roughly turbinate shape characteristic of the mature zoosporangium. But before the parasite begins to expand, it generally penetrates straight into the tissues until it has reached the vicinity of a vascular bundle. The final size of the cyst is roughly proportional to the length attained by the germ tube, but of course the relation is somewhat accidental, since it is the stronger bundles capable of supplying more abundant food which are the more deeply buried. In the leaves the distance is approximately 100 μ, while in the stems, where the vascular bundles are relatively deeply buried beneath the cortex, a length of 300 μ or more is frequently attained (fig. 14). It thus happens that size is no criterion of the age of a cyst, some uninucleate cysts being much larger than some which are far along in division, as shown by figs. 12 and 26, which are drawn to the same scale.

Sometimes, while still in the tubular condition and usually before full size has been reached, a characteristic plug is formed at the mouth of the zoosporangium. In all but the youngest stages this is the most convenient character for distinguishing the zoosporangia from the resting spores, since the latter never develop a plug. But the plug is subject to great variations in size, and in rare instances may never develop at all. The most typical form is a solid top-shaped mass which stains deeply and uniformly throughout (figs. 14, 15, etc.). Often it is a hollow, bell-shaped structure (fig. 21), as figured by LAGERHEIM (see above, p. 136). In some instances such bell-shaped plugs were found to be perforated so as to place the interior of the cyst in open communication with the outside. Some solid plugs were observed which stained lightly, except on the lateral edges (fig. 24), giving the appearance of bell-shaped plugs which had been later filled up. In many cases the

plug is secondarily surrounded by several concentric layers of material, evidently laid down at intervals. Such plugs show great variation in appearance (figs. 17–20), presumably on account of variations in the conditions of deposition.

As in the resting spore, the protoplast is at first highly vacuolate, consisting of a peripheral layer of cytoplasm connected with the central mass about the nucleus by radiating strands. As growth proceeds, the cytoplasm becomes more abundant in proportion to the vacuoles, but the zoosporangia always have larger vacuoles than the resting spores. Sporangia of different ages, however, vary considerably in this regard. The larger cysts usually have larger vacuoles than the smaller. In later stages there is always one large vacuole which occupies the upper half of the cyst, the protoplasmic contents, except for a thin peripheral layer, being confined to the basal portion, as shown in the figures. The numerous rhizoids which are put out from the base are like those of the resting spore.

The cysts reach full size before there is any indication of division. But when division commences, the binucleate, tetranucleate, and later stages follow each other in rapid succession (figs. 22–28), until a large but variable number of nuclei have been formed. Upon completion of the period of nuclear division, segmentation occurs and zoospores are produced. The coenocytic cysts are comparatively rare. Never, even in the most favorable material, do they approach in abundance the primary cysts or those in which segmentation is complete.

The shape of the cysts seems to be determined largely by accidental variations in the compactness of the tissues in which they lie. The penetrating germ tubes follow to a large extent the path of least resistance. This sometimes leads them to spread out in the tissues (fig. 2), and causes considerable irregularity in the form of the mature cyst.

In those cysts which have abundant starch, clear spaces, roughly corresponding in size and shape with the primary nuclei, persist for some time after division (figs. 22, 57). Similar appearances are found sometimes in the telophases of the later mitoses (fig. 66). These are not vacuoles, as might at first appear from contrast

with the starch-filled cytoplasm surrounding them, but are occupied by cytoplasm similar to that of the remainder of the cyst, except that it is free from starch. This condition endures for a variable period; it sometimes disappears during the binucleate stage (cf. fig. 23), and sometimes persists into the octinucleate stage (fig. 24).

The nuclei of the early stages of the coenocyte tend to remain in the central position originally occupied by the primary nucleus, but later scatter, finally becoming evenly distributed through the cytoplasm. The period at which they disperse varies, as would be expected. One case was found in which they were still closely bunched in the 16-nucleate stage (fig. 25), but they are usually dispersed a little before that time.

SEGMENTATION.—On account of certain apparently conflicting processes observed, the writer has not been able to satisfy himself altogether concerning the mechanism by which the coenocytic cyst is cut up into spores. The account here given is therefore somewhat tentative.

During the last mitoses in the sporangium, a change seems to come over the protoplasm of the coenocyte. Up to this time the nuclei have apparently lain freely in the common cytoplasm without any tendency to form separate cells. But during these mitoses the cytoplasm appears to contract around the spindles and to draw up closer to them, so as to leave vacuoles in the intermediate spaces (fig. 30). These vacuoles, surrounding, as they do, the separated masses, often resemble cleavage furrows cutting the coenocyte up into individual cells. The cytoplasmic edges of the segments do not present the sharp clean outlines seen in progressive cleavage, however, but appear more or less irregularly frayed, and frequently cytoplasmic strands cross the vacuoles and connect adjacent masses.

These connections would seem to put aside any interpretation of the process as due to cleavage furrows, but one cyst was observed in which the margins of the individual masses were clear and sharp, without any bridges across the furrows (fig. 29). This case was difficult to interpret otherwise than as progressive segmentation by cleavage furrows.

This cytoplasmic contraction appears to be a universal occurrence, having been seen in all of the numerous cysts of this age observed. Nevertheless, in the writer's judgment it is not to be interpreted as segmentation. That appears to be a distinct process of a different nature. Since the preliminary contraction occurs during mitosis, it gives rise not to uninucleate but to binucleate segments. No indication of a constriction separating the daughter nuclei was seen in the telophases observed (figs. 63–65). The steps connecting this condition with what I take to be true segmentation could not be made out, but it would seem probable that the contraction disappears after mitosis is complete and the protoplasm of the cyst again becomes a continuous coenocyte. It will be understood that a regressive change of this character would be difficult to demonstrate except in living material, which in *Rhodochytrium* is too thick and too deeply pigmented to permit the observation of details of this sort. If the zoospores were always the same size, or if segmentation always occurred after a given number of nuclear divisions, it might be possible to recognize those cysts which had passed through their last mitosis and were ready for the final segmentation, but both the size of the zoospores and the number formed in different sporangia vary to such an extent as to make it impossible to distinguish those sporangia which have completed the cycle of mitosis from those which have not.

But whether the cysts again become continuous coenocytes or not, there is another sort of cleavage, which I take to be true segmentation, that appears to delimit the spores without reference to the separations brought about during the preliminary contraction. This occurs by the precipitation of membranes around the protoplasmic units (fig. 31). Each nucleus with its quota of cytoplasm is cut off from the rest by a membrane which appears within the strands of cytoplasm after the fashion of free cell formation in the endosperm of a seed plant. The membranes of the protospores are very delicate, but the method of their formation seems to be clearly indicated in the preparations. If one observes a protospore which is not yet completely surrounded, the terminal portion of the advancing membrane will appear simply as a heavy

strand of cytoplasm (fig. 31, *a*). The spores seem to round off soon after their membranes are laid down, presenting as they do so somewhat the appearance of bodies being divided by advancing cleavage furrows. Observation of the terminal portions of the apparent furrows shows, however, that they merely separate spore membranes already formed by precipitation within the cytoplasm. This is made especially clear at the angles of the protospores, where the membranes frequently cut across the corners, leaving small portions of the cytoplasm which do not enter into the formation of any spore (fig. 31, *b*).

MATURATION OF ZOOSPORES.—Although the protospores quickly round off and separate from each other, they remain in the position occupied before segmentation. Consequently the mass of young spores retains the shape of the coenocyte from which it was derived, leaving the central vacuole unoccupied as before segmentation, as in fig. 28, which shows the condition of the great majority of the segmented cysts observed. In such sporangia the young spores are usually regular ovoid cells (fig. 33), without the differentiation of parts characteristic of the mature spore. Only rarely were fully matured zoospores which had moved out into the cavity of the cyst found in the sections studied. In such ripened spores there is a conspicuous differentiation into anterior and posterior ends (figs. 35, 36). In the posterior end is collected the larger part of the cytoplasm with the starch grains, if any be present, while the anterior end appears highly vacuolate in fixed preparations on account of the removal of the pigment which occupied it during life.

In no case was I able to assure myself that cilia were present in the section studied, although I thought I saw them several times. This was probably due to imperfect fixation, since the chromacetic acid used is not as well adapted for preserving such structures as some killing fluids which might have been used had it been possible to experiment on the ground. In zoospores fixed in osmic fumes, after liberation the cilia were of course clearly shown (fig. 37), and in these, as well as in many of those on the sections (fig. 36), there was a conspicuous deeply staining body at the base of the cilia such as has been found in zoospores of many other forms. In many of the spores, especially those a little over-

stained, one or sometimes two delicate connections could be seen between this basal body and the nucleus (fig. 34). The origin of the basal body was not made out. Apparently it appears only during the maturation of the spore, for it was not observed in earlier stages (figs. 32, 33).

The primary nucleus

Although the youngest cysts observed are many times larger than the zoospores from which they originated, their nuclei show comparatively little enlargement. But they differ somewhat in character from the nuclei of the zoospores in that the concentration of the chromatin, which, as shown above (figs. 32–37), begins in the maturing zoospore, has been completed, forming the karyosome, which is the most conspicuous element of the nucleus. But the karyosomes of the young cyst have not acquired the character of the later nucleoli. From the irregularity of their shape they appear to be merely plastic masses of chromatin (fig. 42). They soon take on the definite spherical form of mature nucleoli, and at the same time probably become firmer, inasmuch as in the later vacuolate stages the rind is strong enough to retain its shape after most of the contents have been withdrawn. The linin reticulum seen in the sporangial segments probably persists on the periphery of the nucleus in the youngest stages, but it loses its affinity for stains and is exceedingly difficult to see satisfactorily. All that can be made out with certainty in most of the nuclei is a few delicate linin strands stretching from the karyosome to the nuclear membrane (figs. 1, 13), or, in optical section, a number of peripheral granules (figs. 2, 12), which probably represent cross-sections of the similar strands that compose the reticulum, but are too faintly stained to be visible in surface view.

No differences between the nuclei of the incipient zoosporangia and of resting spores were detected. From the youngest stages on they undergo the same development, which in one case leads to mitosis and in the other to shriveling preparatory to the long dormant period.

The most conspicuous of the changes in the nucleus is its increase in size. From 4 or 5 μ it grows with the cyst until it may reach

the enormous size of 50–60 μ (figs. 15, 45). This size, however, is attained only in the largest zoosporangia. The nuclei of the resting spores are never so large as those of the zoosporangia, which themselves vary greatly, being roughly proportional to the cysts in which they occur. In extremely small cysts the nucleus may never exceed 15 μ, though few are smaller than 20 μ at maturity. In the largest nuclei the increase in volume during growth is almost 10,000 fold. There are but few organisms in which any single nucleus grows to such an extent without division, but *Rhodochytrium* is by no means unique in this respect. In *Synchytrium*, by reason of the minuteness of the zoospores, the increase is very much greater, amounting sometimes to 50,000 fold. In some of the cycads, especially *Dioon* (CHAMBERLAIN **5**), the increase in volume must be nearly as great, since the mature nuclei reach 500–600 μ in diameter. The nuclei of some animal eggs, for example *Dytiscus* (DEBAISIEUX **7**), also show great increase in volume, but not so much as in the plants just cited.

For the study of the vacuolation of the nucleolus, *Rhodochytrium* and *Synchytrium* probably afford better opportunities than any other organisms, although an analogous process occurs in many plants. Occasionally in *Rhodochytrium* a single central vacuole appears to increase in size until only a thin rind of stainable substance remains. In other cases the whole nucleolus becomes honeycombed with numerous small vacuoles (fig. 46), which later coalesce (fig. 47) into a large central cavity (fig. 44), which continues to increase in size until finally the old nucleolus, originally a karyosome, becomes a plasmosome, collapses (fig. 45), disintegrates, and finally disperses in the cytoplasm during mitosis.

Intimately connected with the history of the nucleolus, and in many ways perhaps even more interesting, is the behavior of the chromatin. As may be seen from figs. 2, 12, 42, the whole of the chromatin is at first concentrated in the karyosome, and from it all of the chromatin of the primary nucleus is derived. While the nucleus is still comparatively small, vacuoles begin to appear in the center of the karyosome (figs. 3, 13, 14), and the characteristic irregular masses of chromatin begin to fill the nuclear cavity. As

in *Synchytrium*, these are most abundant in the vicinity of the nucleolus (karyosome), frequently touching it. Closer examination will often reveal many in the act of budding out from it (figs. 43, 47). During the growth of the nucleus there is, of course, an enormous increase in the amount of chromatin it contains. This increase of the chromatin probably takes place both in the nucleolus during its growth and in the free chromatin of the nuclear cavity. But the withdrawal of the chromatin from the nucleolus must be more rapid than its formation therein, since the vacuolation of the nucleolus increases with age. The linin reticulum, which, as has been seen, loses its affinity for stains in the young cysts, never reappears in the primary nuclei. The chromatin, as it is withdrawn from the karyosome, does not seek the nuclear membrane, but is distributed through the nuclear cavity. In the early stages of growth the chromatin spherules are often connected by indefinite strands of linin, which anastomose to some extent through the nuclear cavity (figs. 3, 14, 44). But in many of the nuclei (fig. 43) such linin connections never appear, and in any case they disappear before the nucleus reaches its full size. In mature nuclei (fig. 45) the chromatin appears as amorphous, almost flocculent, spheroidal masses scattered through the nuclear cavity, singly or in loose chains. The amount of chromatin and the size of its masses vary considerably in different nuclei. In some cases there are relatively few large globules, while in others the chromatin, in a comparatively fine state of division, almost fills the cavity of the nucleus. The small intensely staining granules, which are so conspicuous against the membranes of the primary nuclei of *Synchytrium*, are seldom observed in *Rhodochytrium*, but in some instances (fig. 57) were as prominent as in *Synchytrium*.

The peculiarities of the primary nucleus characterize to a large extent the nuclei of the binucleate and tetranucleate stages, but gradually disappear as the nuclei become smaller, until, from about the 32-nucleate stage on, the nuclei resemble those commonly found in other organisms. Except in the very latest stages, however, both the chromatin granules and the linin connections are coarser than in most nuclei.

Mitosis

There are two types of mitosis in *Rhodochytrium*. The first type occurs in the earlier divisions of the zoosporangium, while the second is found in the last divisions before sporulation. They are not, however, to be considered as distinct, for they merge into each other.

No evidence of a reduction division was found. Nowhere were nuclei seen in fours, as would be expected after reduction; and while the chromosomes are difficult to count accurately, I feel sure that their number was approximately the same in the last divisions as in the primary mitosis. They are extremely difficult to count, however, because they are usually close together and often surrounded by starch grains. For this reason it was not possible to count the chromosomes of as many spindles as would have been desirable, nor to insure exactness in the cases counted. In all of the cases where counting was attempted, however, the number was no smaller than 8 nor larger than 10.

The assembling of a series of stages of mitosis is an exceedingly tedious task. As already stated, coenocytic cysts of any sort are comparatively rare. Those in mitosis are of course rarer still. It is doubtful if one cyst in a thousand of those observed showed dividing nuclei. The anaphases and telophases are particularly difficult to find. It was not possible, therefore, to examine a large number of figures of the different stages. But inasmuch as the spindles found form a concordant series, it is believed that the account given accurately describes the process.

MITOSIS OF THE FIRST TYPE.—The typical mitosis of the first type is the division of the primary nucleus, but the second and third mitoses are so similar that for purposes of description they may be said to be identical. Drawings from all of these have been used in the plates indiscriminately, but they may be identified, if desired, by the explanation of the plates.

Spindle formation.—The first indication of approaching mitosis consists in the appearance of kinoplasmic fibers among the masses of chromatin in the nucleus. The change shown in fig. 48 is so slight that it would hardly have been detected had not the other nuclei of the cyst been already far advanced in spindle formation,

thereby drawing attention to the laggard. Coincident with the appearance of these kinoplasmic fibers the chains of chromatin usually break up, and the individual masses become more definitely spherical, karyosome-like structures. In a nucleus a little further advanced the fibers have become more abundant and permeate all parts of the nuclear cavity (fig. 49), and on some of them are seen small deeply staining granules whose origin, fate, and function are not altogether clear to me.

From the very first the position of one of the poles of the future spindle can be recognized in the focus of certain of the fibers (figs. 48–50). Curiously enough, however, the other pole does not seem to appear until somewhat later, so that the young spindles show a considerable difference in the two poles, one being more fully formed than the other (fig. 51). This is such a peculiar phenomenon that one is strongly inclined to believe, when he finds such a nucleus, that he has overlooked the opposite pole on another section (most of the spindles are of course somewhat oblique), but careful search almost invariably failed to reveal it. Fig. 50, which is a sagittal section of a primary nucleus, shows perhaps the extreme of this condition; notwithstanding the strong development of kinoplasmic fibers in the part of the nucleus drawn, they were entirely absent from the other parts. It is quite possible that the spindles seen in these stages were unusual, but the occurrence of the unipolar condition in different pieces of material killed in different years has convinced me, against my prejudices, that this is a normal and usual method of spindle formation.

Such a drawing as fig. 50 resembles the prophase in the Ascomycetes, in which the linin strands containing the chromatin radiate from one side of the nucleus. There are, however, important differences between the two. The polarity of the ascomycetous spindle is determined by the presence of centrosomes attached to the nuclear membrane, but in *Rhodochytrium* no centrosomes are visible, and the pole does not necessarily touch the nuclear membrane at all. The origin of the bipolar condition is entirely dissimilar. In the Ascomycetes the two centrosomes, derived from the fission of one, separate and migrate to opposite sides of the nucleus, each carrying with it its quota of fibers with attached

chromatin. But in *Rhodochytrium* the second pole is formed, like the first, by the convergence of certain fibers to a point. In nuclei a little older than that shown in fig. 50, some of the kinoplasmic fibers can be seen to intersect at points more or less directly opposite the first pole. There are usually two or three such points (fig. 51), from each of which a few fibers radiate. In later stages one of these focal points becomes more prominent than the others, until ultimately it becomes the second pole of the spindle, as prominent and definite as the first.

In the fully formed spindle the larger proportion of the fibers of course stretch from pole to pole, but in the early stages the rays from each pole appear as an independent fascicle radiating from the focus, with little regard to the position of the opposite pole. The vestiges of this condition may be seen in fully formed spindles, in which many of the acicular mantle fibers stretch straight by the equator of the spindle, intersecting those from the opposite pole (figs. 52–54). Not infrequently a few fibers center in the pole and do not enter into the formation of the spindle, but radiate into the nuclear cavity. In one instance such radiations were so numerous as to give the appearance of a conspicuous aster (fig. 53). But comparison with the opposite pole shows that the effect here produced is largely accidental. Nothing similar was seen elsewhere.

Chromosome formation.—The differentiation of the chromosomes, in my material, is a much more difficult matter to follow than the formation of the spindle. Of the masses of chromatin which are distributed throughout the nuclear cavity, part remain free and part become connected with the developing spindle fibers. In addition to these, some of the spindle fibers, especially in the early stages, are studded with smaller chromatic granules whose significance, as stated above, is obscure to me. At one stage of the investigation I was inclined to believe that these were used in the formation of the chromosomes, but further observation has led me to the conclusion that it is the large chromatin masses which give rise to the chromosomes. Whether the chromosomes are derived exclusively from the latter is not certain, but such figures as no. 51 show at least that some of them are utilized in chromosome formation.

The formation of the chromosomes, though it presents certain striking peculiarities, conforms in its essential features to the process usually found in dividing nuclei in other organisms. As is not unusual, spindle formation and chromosome formation, being in a sense unconnected processes, may go on side by side with a certain degree of independence, so that in two nuclei of the same age one may have the more mature spindle, while the other has advanced further in chromosome formation (figs. 52, 53).

Spirem formation will be understood by a glance at fig. 51. Between those chromatin spherules which lie in the equatorial region of the nascent spindle there arise connecting bands of linin, forming an irregular spirem. At first the stains differentiate the chromatin and the linin elements, but in later stages the spirem stains homogeneously like other spirems. In the beginning its position may not be so definite, but as it contracts it comes to lie wholly within the spindle (fig. 52). After some further contraction it segments into chromosomes in the usual way (figs. 53, 54).

Only a small portion of the chromatin of the primary nucleus is utilized in the formation of this spirem. On the dissolution of the nuclear membrane the remainder is cast out into the cytoplasm. There is no indication of any difference between those chromatin masses which are cast out and those which enter into the spirem, nor of any principle of selection other than that occasioned by the mere position of the masses which are utilized. Sometimes the masses of discarded chromatin persist for some time as deeply staining globules in the cytoplasm (fig. 22), but more often they lose their affinity for stains before the nuclear membrane breaks down and cannot be followed in later stages.

During metaphase the spindle, which previously may have been shorter than the diameter of the nuclear cavity in which it lay (fig. 53), begins to elongate, piercing the membrane (fig. 54), and later, as the membrane weakens preparatory to dissolution, distorting the nucleus (fig. 55). The only anaphases seen were of the first type, occuring in the fourth mitosis. Apparently the chromosomes are drawn away from the equator in the usual way (fig. 56). No stages showing the formation of the membranes of the daughter nuclei were seen in spindles of the first type, but two

recently divided binucleate cysts were found. Their chromatin strands (fig. 57) still showed by their orientation the position of the chromosomes from which they had been derived. As stated above, the position of the mother nucleus is still clearly indicated by a starch-free area in the cytoplasm.

No centrosomes or asters, except the pseudoaster above noted, were seen in connection with any of the spindles. The poles are very sharp, without any surrounding zone of denser cytoplasm in which a centrosome might have been concealed. There is no indication that astral bodies have any part in the formation of the nuclear membrane, as in *Synchytrium decipiens*.[3] While but very few of the critical stages were seen, it seems evident that, if there were any such conspicuous asters as in that plant, they would certainly have appeared in the preparations studied.

In the intermediate mitoses, spindle formation conforms in a general way to that in the primary nucleus, but the metaphases (figs. 58, 59) are so different that at first sight they would seem to be of an entirely different type. The differences, however, are not so great as would appear. In the smaller nuclei nearly as great an amount of chromatin is used in the formation of the chromosomes as in the larger. Their spirems are therefore much larger proportionately, and, instead of lying within the spindle, stretch nearly across the nuclear cavity. Sometimes such spindles show a considerable amount of chromatin which is not utilized in the formation of the chromosomes, but is cast out, as in the earlier divisions. Frequently, however, all of the chromatin goes into the spirem (fig. 59). The karyosome, which is so strongly developed in the primary nuclei, becomes gradually less and less prominent in later nuclei, until in the many-nucleate cyst the chromatin assumes the condition of a typical reticulum, although it is not finely divided, but remains in rather large masses which are connected by coarse linin strands (figs. 27, 29). In consequence of the different dispositions of the chromatin in these nuclei, the residual chromatin cast out during their mitosis does not take the form of large spherules, but is finely subdivided (fig. 58). Such a condition was also seen

[3] *S. taraxaci* is without karyodermatoplasts according to the recent results of BALLY (Jahrb. Wiss. Bot. **50**: 110. 1911).

in one primary nucleus, in which case the residual chromatin was much more abundant than in the smaller nuclei.

MITOSIS OF THE SECOND TYPE.—The second type of mitosis is limited to the last few divisions before sporulation. Unfortunately nearly all of the mitoses of this type that were found occurred in cysts packed full of starch, which greatly interfered with observation.

The difficulties occasioned by this cause were especially serious in studying the prophases. In the cyst from which the figures of prophase were taken, all stages of prophase were certainly present, but could not be made out satisfactorily. The nuclei of the upper half of the sporangium had already passed into the metaphase, while those in the rhizoidal end were still in the vegetative condition (fig. 60), and above them all transitions to metaphase were present. As far as could be determined, these prophases were similar to those of the smaller nuclei of *Synchytrium*. A spirem is formed which in this case involves but little change from the vegetative condition. This then shortens and thickens until it comes to occupy only the equatorial region of the nucleus (fig. 61). The spindle then appears, whether as a new formation or as a metamorphosis of linin strands as in *Synchytrium* could not be determined.

The chromosomes in this type of mitosis are small and spherical (fig. 62), but apparently stretch out somewhat in fission, for at the poles in telophase they are distinctly oblong (fig. 63). In early telophase they are bunched together in a compact mass resembling the familiar "daughter star," but later begin to spread out (fig. 64) and assume irregular shapes (fig. 65), while vacuoles of karyolymph begin to appear among them, soon producing the characteristic vegetative nuclei (fig. 66). As may be seen from the figures, these stages are practically similar in all respects, save in the absence of cell plate, to the familiar anaphases and telophases of the higher plants.

Amitosis

Amitosis, which forms such a conspicuous feature of the cytology of *Synchytrium*, is almost absent from the zoosporangia of *Rhodochytrium*, or at least from the material studied. The nuclei of a few

cysts, however, are in such a condition that it seems hardly possible to interpret them as sister products of mitosis. Their chromatin assumes the condition of an extremely long and complicated spirem, which winds not only around the surface of the nucleus but fills its cavity (figs. 67, 68). Their shape is extremely irregular. The largest have developed pseudopodium-like outgrowths, which appear to have been constricting off into daughter nuclei. With these large nuclei are a number of small ones, apparently the results of the process. While the mere irregularity in the outlines of these nuclei would not in itself be conclusive evidence that they were dividing amitotically, the great diversity in the sizes of adjacent nuclei would be difficult to account for on any other hypothesis. For in *Rhodochytrium*, as in coenocytes generally, the mitoses are simultaneous, and the daughter nuclei are of approximately equal sizes (figs. 22–27). It is evident that such a process could not normally give rise to irregularities in either number or size of the resultant nuclei.

There is no indication, however, that amitosis is a normal process in the zoosporangia of *Rhodochytrium* as in *Synchytrium*. It gives rather every indication of being a pathological phenomenon.

Cytological comparisons

PRIMARY NUCLEUS.—The primary nuclei of *Rhodochytrium* are certainly very peculiar; indeed, if the cytology of *Synchytrium* were not known, we should say they were unique. But when mature they are strikingly similar to those of *Synchytrium*, or at least to those of *S. decipiens* and *S. puerariae*. The conditions sometimes found during the early portion of the growth period, however, are not paralleled in *Synchytrium*. The early stages of *Synchytrium* are very similar to the mature nuclei, but in the young nuclei of *Rhodochytrium* the chromatin spherules are often suspended on anastomosing strands of linin within the nuclear cavity (figs. 3, 14, 44). This condition is evidently less removed from the typical peripheral chromatin-linin reticulum of most nuclei than are the mature nuclei or those of *Synchytrium*.

The irregular masses of chromatin in the primary nucleus of *Synchytrium* are termed by KUSANO (18) secondary nucleoli. He

shows, what I have myself observed, that they may pass through a process of vacuolation accompanied by the extrusion of chromatin analogous to that of the primary nucleolus. In *Rhodochytrium* such secondary vacuolation occurs but rarely, though some of the largest chromatin masses may break up in this way (fig. 49). But, as was shown in the account of mitosis, a large proportion of the chromatin spherules suffer the same fate as the old nucleoli, primary and secondary, of *Synchytrium*, namely dissipation in the cytoplasm. There is, moreover, a great variation in the size, composition, and behavior of the secondary nucleoli in *Synchytrium* (see KUSANO **18,** p. 94), some of them (the earlier and smaller) being almost, if not entirely, pure chromatin, and undergoing but little change in preparation for mitosis; while others (the later and larger) are plasmosomes with but little chromatic material. There is, therefore, no question but that the chromatin masses of *Rhodochytrium* are homologous to secondary nucleoli, but it does not seem advisable to use that term in describing them, since there is no distinction between those which form the chromosomes of the spindle and those which perish.

MITOSIS.—The first mitoses of *Rhodochytrium* and *Synchytrium* are not so similar as are the primary nuclei, but they are of the same general type. Although very different from those found in most organisms, the first mitosis of *Rhodochytrium*, like the vegetative condition of the primary nucleus, is not so widely aberrant as that of *Synchytrium*. Neither STEVENS nor KUSANO was able to obtain an altogether satisfactory series of the prophases of the primary mitosis, and their figures do not supplement each other, but conflict to a certain extent. Both observed, however, a marked and peculiar production of fibers, STEVENS through the whole cavity of the nucleus, and KUSANO especially in the region of the old nucleolus after the dissolution of the membrane. While the conditions found by these writers in *Synchytrium* differ greatly in detail from those in *Rhodochytrium*, the fibers would seem to be comparable to those seen in the early prophases of the present plant. If this interpretation is correct, the fibrous stage in *Synchytrium* is not to be homologized with a spirem, but is rather a phase of spindle formation. The differentiation of the chromosomes,

which neither of these writers was able to observe, would on this assumption be a distinct process. While it cannot be predicted that in the differentiation of the chromosomes *Synchytrium* will be found to resemble *Rhodochytrium*, it is clear that in the formation of the spindle there is considerable analogy.

While the metaphases, and probably the prophases as well, of the second type of mitosis are similar to those of *Synchytrium*, this has no particular significance, since they present no peculiarities, but are similar to those of many other organisms. The telophases, however, differ considerably from those of *Synchytrium*, both in general form and in the absence of the conspicuous kinoplasmic asters, *karyodermatoplasts*, which in *Synchytrium decipiens* and *S. puerariae* form the nuclear membranes of the daughter nuclei. These structures remain, therefore, peculiar to these species.

KARYOLYMPH.—The large primary nuclei, of course, are cut into several sections by the microtome. The central section of such a nucleus presents an appearance which would hardly be recognized by the uninitiated, for it looks at first sight like a hole in the cytoplasm of the parasite. It is surrounded, however, by the nuclear membrane and contains some of the amorphous masses of chromatin and perhaps a part of the nucleolus. But sometimes the whole nuclear cavity is filled with a frothy mass similar to that noticed by KUSANO in *Synchytrium* after fixation with Keiser's fluid. It appears to be, what KUSANO interpreted it, a precipitation from karyolymph. I have not figured it because it is inconstant in occurrence and imperfectly understood.

It should be noticed here, however, that the karyolymph may very likely play a much more important rôle in cell physiology than is at present assigned to it by cytologists. It is dismissed with a sentence in such texts as WILSON'S *Cell*, because our knowledge of it is practically nil. Yet, ignorant as we are, a little reflection will convince us that it must be of some consequence to the cell. On the amount of karyolymph depends the size of the nucleus, for it is in reality merely a vacuole of karyolymph around which is stretched the chromatin reticulum. It is a well-known fact that by some means the size of this vacuole is maintained with slight variation in the cells of a given tissue. We know further that when

by any abnormality the amount of chromatin is increased, as when a nucleus passes through the prophases of mitosis but fails to divide, the karyolymph is proportionately increased. The characteristic phases of the nucleus, vegetative and mitotic, are marked off from each other principally by the appearance and dispersal of the karyolymph. Indeed, it is a general rule that whenever the karyolymph is absent, the anabolic activity of the cell is suspended. The characteristic condensed condition of sperm nuclei is another illustration. The shriveling of the nuclei of the resting spores in *Rhodochytrium* above described is due to the partial disappearance of karyolymph when growth ceases and the dormant period is entered upon.

SEGMENTATION.—In regard to the process of segmentation, the uncertainties encountered in *Rhodochytrium* are largely duplicated in *Synchytrium*. HARPER (**12**) reported that segmentation occurs by the formation of cleavage furrows, which begin to penetrate the cytoplasm at a relatively early stage in the multiplication of the nuclei. KUSANO (**18**) found that while some cysts undergo progressive cleavage, as described by HARPER, others show simultaneous segmentation by the precipitation of membranes around the segments. My own observations, like KUSANO'S, showed both of these methods of segmentation, but in my material the progressive cleavage described by HARPER was infrequent. The apparent duplication of segmentation recalls the double contraction reported in various phycomycetes and certain algae, such as *Hydrodictyon* (KLEBS **14**, TIMBERLAKE **30**). But it is not easy to correlate the accounts of observations on living and on fixed material, and for that reason the writer finds himself unable to interpret the phenomena satisfactorily.

Alga or fungus?

Having examined the morphology and cytology of the plant, we may proceed to consider its relationships. Since it is an obligate parasite without chlorophyll, one naturally wonders how it was ever referred to the protococcoid algae. On a superficial examination certainly, it would appear that the plant is no alga but a chytrideaceous fungus. The first question that arises, therefore, is

whether *Rhodochytrium* is an alga or a fungus. As will be seen, the answer depends not so much upon any interpretation of the facts of the case, as upon the point of view of the student.

Among the Chytridiales, *Entophlyctis*, of the family Rhizidiaceae, is strikingly similar to *Rhodochytrium* in gross morphology. Both are characterized by an external button connecting by a narrow neck with the main body of cyst. The rhizoidal system, if not exactly of the same appearance in the two cases, is of the same type, and the differences may be supposed to be due to the character of the substrata, which in one case is the soft protoplast of an alga and in the other the tough vascular bundle of a seed plant. The life cycles are identical; both start from a free swimming zoospore that penetrates the host, giving rise to an internal ampulla which on maturity becomes either a resting spore or a zoosporangium. Altogether *Entophlyctis* is so similar to *Rhodochytrium* that the comparison is exceedingly suggestive.

Nevertheless, there does not seem to me to be any good reason for connecting *Rhodochytrium* and *Entophlyctis*. The comparative anatomy of the Rhizidiaceae would seem distinctly to forbid such an idea. Within the family Rhizidiaceae there are apparently all transitions from purely epiphytic parasites with as little penetration as possible, to complete endoparasites. At the beginning of the series may be placed *Rhizophidium brevipes*,[4] which barely penetrates the wall of its host, without putting out any rhizoids to gather nutriment. Further stages are shown by various species of *Phylactochytrium*, which not only have extensive rhizoids, but develop a small basal portion of the plant body itself within the host. In *P. equale* the internal portion of the body becomes as large as the external. From this condition it is an easy step to *Entophlyctis* by the enlargement of the internal portion at the expense of the external, with consequent transference of the sporogenous function. This has every appearance of being a natural phyletic series. In it the parasitic mode of life would appear to

[4] *Harpochytrium* is even more surely an epiphytic parasite, since it does not penetrate its host at all, being merely attached to its wall; but it is not used in the present comparison because its relationships have been subject to some difference of opinion among different observers. WILLE (33), for example, believes that it is a colorless member of the Protococcoideae.

have been developed from an epiphytic ancestry, while endophytism did not appear until later.

In contrast with this group, *Rhodochytrium* seems to have been derived from organisms which acquired the endophytic habit of life before any real dependence on their hosts was established.

Moreover, the zoospores of *Rhodochytrium* appear to differ fundamentally from those of the Chytridiales. In most of the latter there is but one flagellum, which is often trailed along behind and imparts a weak jerky motion to the spore. In the genera with biflagellate zoospores the flagella, in most cases at least, are of the same type, and usually spring from different portions of the body.[5] Sometimes also the spores put out pseudopodia and move about in amoeboid fashion. In *Rhodochytrium* the zoospores are capable of no such motion, but maintain the integrity of their shape with slight variation throughout their period of activity. The cilia, which are anterior, are more highly specialized structures and maintain a rapid vibration which propels the spore with the steady motion characteristic of algal zoospores in general, to which those of *Rhodochytrium* correspond in every important particular, save in the absence of chlorophyll.

But the nature of the parasitism of *Rhodochytrium* indicates a very considerable degree of departure from the algae. An obligate parasite which has established definite relations with specific hosts, even though its different races show no morphological modification, is certainly far from a typical alga. The loss of plastids is an important characteristic of the fungi, but the presence of starch grains looks back toward the algae. Though starch has been reported in several fungi, and some of them contain certain carbohydrates which give the starch reaction with iodine, such as starch cellulose ("lichenin"), there is no well authenticated instance of the occurrence of definite grains of starch in any undoubted fungus.

Turning now to the algae along the lines suggested by LAGERHEIM'S paper, we find among the protococcoid algae a number of

[5] In a paper to be published almost concurrently with this (Ann. Botany, January 1912), the proof of which I have seen through the kindness of the author, Dr. J. T. BARRETT, it is shown that the zoospores of several species of *Olpidiopsis* have two flagella *springing from the same point*, while other species of the same genus are reported as uniflagellate.

very interesting endophytes or "*Raumparasiten*," which have been made known principally by the researches of KLEBS (**13**). The climax of this series is found in *Phyllobium dimorphum*, which penetrates dying leaves of *Lysimachia nummularia*. Its adult body is strikingly similar to that of *Rhodochytrium*. There is a long empty neck, with an external cellulose button connecting the internal cyst with the wall of the zoospore from which it developed, just as in *Rhodochytrium*. In its most typical development this plant is confined to the vascular bundles of its host, into which it penetrates very much as does *Rhodochytrium*. It sends out, moreover, numerous interlacing rhizoids, which follow along the bundles for considerable distances, and even extend up their branches. On germination the resting cysts give rise to biciliate zoospores which conjugate as in *Rhodochytrium*, except that there is a slight sexual differentiation, microzoospores and megazoospores being formed in different cysts. The cysts and the zoospores have abundant chlorophyll, but haematochrome is also present in considerable amounts in some stages of the life cycle. Little is known of the finer structure or cytology[6] of this plant, but, so far as one can judge from the evidence available, it is remarkably close to *Rhodochytrium*. The most important difference between them would seem to be the presence of chlorophyll in the one and its absence in the other. OLTMANNS (**21**, pp. 322 ff.) believes that these forms belong to a natural series. He agrees with LAGERHEIM that *Rhodochytrium* is an alga, saying "while the first named genus [*Phyllobium*] cannot be considered more than an endophyte, as we have already clearly demonstrated, *Rhodochytrium* is one of the rare examples of an alga which has lost its chlorophyll on account of parasitism."

It will be seen, therefore, that the decision as to whether *Rhodochytrium* is an alga or a fungus depends upon the criteria by which the line between them is to be drawn. If the question is to be settled by definition, we should follow VUILLEMIN (**32**) and call it a fungus, for it would be very difficult to frame a definition of the fungi which would exclude *Rhodochytrium*. This position is also

[6] OLTMANNS states, on the basis of unpublished observations by GRUEBER, that the cyst is uninucleate.

taken by LINDAU (**20**), who excludes it from the algae on account of the absence of chlorophyll. If, on the other hand, the matter is to be decided by the relationships of the plant, it is clear that since its nearest affinities are with undoubted algae, *Rhodochytrium* must be considered an alga. It is not a matter of great consequence whether such an organism is considered a fungus or an alga, so long as its real affinities are recognized. But in the case of *Rhodochytrium* it will probably be more convenient to consider it with the algae than with the fungi, since it can be satisfactorily approached only from the algal side.

Evolutionary inferences

But although *Rhodochytrium* is to be considered the extreme of an algal series and not a near relative of any of the Archimycetes, the phyletic position of the Phyllobiae, taken as a whole, remains to be considered. We have here a series of endophytes culminating in a colorless parasite. Does this line of evolution end blindly, or do these forms furnish the clue to the origin of some fungal group? Nearly forty years ago, before *Phyllobium*, *Rhodochytrium*, and *Endosphaera* were discovered, COHN (**6**) recognized the general similarity of his newly discovered *Chlorochytrium* to *Synchytrium*, and suggested that the two were phylogenetically connected.

There are now known far more points of similarity in gross morphology between the different genera of the Phyllobiae and *Synchytrium* than those which induced COHN to make the comparison. Indeed, could one construct a plant with a combination of characters from the different genera, he would have a very satisfactory transition to *Synchytrium*. Such a hypothetical plant would be an obligate parasite definitely limited to specific hosts, like *Rhodochytrium*. But it would have no rhizoids, retaining rather the simple spheroidal form of *Chlorochytrium* and *Endosphaera*. It would have simultaneous segmentation like *Rhodochytrium*, but the segments would become sporangia rather than zoospores, as in *Endosphaera*, which has substantially the same method of reproduction as *Synchytrium*, except that the swarmers conjugate, while in *Synchytrium* no sexual process is known. It would have lost its plastids, and instead of having chlorophyll

would be pigmented with haematochrome. Should such a plant be discovered, the probabilities are that it would be placed in the Synchytriaceae rather than among the Phyllobiae, where by hypothesis it belongs.

But it must be recognized that the comparison fails utterly at certain points. The germinating zoospore of *Synchytrium* does not form an external button on the surface of its host, and the zoospores are of different types, as shown above. These matters are regarded by some as fundamental criteria of relationship. PETERSEN (23) considers that the presence of an external button in the Chytridiales is clear evidence that they have originated from the filamentous Phycomycetes. But this contention would lose its force if applied to *Chlorochytrium* and *Rhodochytrium*, for these would hardly be regarded by anyone as reduced Siphomycetes. The number of flagella borne by the zoospores is used as a fundamental basis of classification by LOTSY and by VUILLEMIN (32), who regard the genera with biflagellate zoospores as entirely distinct from the other Archimycetes, and classified with them merely because of accidental similarities in form, using as an example *Myzocytium*, which, however, appears distinct from the Chytridiales for other reasons as well. But the Javanese genus *Woroninella* was separated from *Synchytrium* almost entirely on account of the possession of biflagellate zoospores. In all other characters, including the large primary nucleus, it seems to be exceedingly close to *Synchytrium*. Our present information concerning *Woroninella*, which is all contained in a brief description without figures (RACIBORSKI 24), is too meager to enable us to judge whether it is transitional between *Rhodochytrium* and *Synchytrium*. But the description of *Woroninella* goes far to remove those objections to connecting the two that are based on the differences in the zoospores (see also footnote p. 163).

As has been pointed out above in the detailed cytological comparisons, there are some very striking resemblances in cytology between *Rhodochytrium* and *Synchytrium*. Some of these are peculiar to the two genera, being unknown in other organisms. The most conspicuous and perhaps the most significant of these is the enormously overgrown primary nucleus. It is evident that

these are truly unicellular organisms devoid of nuclear as well as cell division until the beginning of the reproductive period. The single cell which composes the plant body does not show any notable specialization in its cell organs, but it reaches a size which is exceeded only by a very few of the largest infusorians, while no nuclei of anything like equivalent size are to be found elsewhere among the Protista. The resemblances in these primary nuclei are not merely superficial, but are emphasized by detailed comparisons of their structure. Though their mitoses differ somewhat in detail, they also are certainly analogous in many respects.

These cytological resemblances, coupled with the general similarity in gross morphology and the tendency toward parasitism so evidently manifest in the Phyllobiae, are certainly very suggestive. It is difficult to imagine that such peculiar cytological features originated independently. If the cytology of the other members of the Phyllobiae and of the genera closest to *Synchytrium* should fall into line with the evidence now available in *Rhodochytrium* and *Synchtrium*, it would make a strong case in favor of a phyletic relationship between the two groups. But it would afford no reason for supposing them *closely* related, for *Synchytrium* appears to occupy an isolated position. The gap which separates it from Phyllobiae would appear to be of ordinal rank, and, at the same time, it is generally recognized that it is far from most other Archimycetes. Nor would it show that *Synchytrium* was derived directly from *Rhodochytrium* or even from Phyllobiae. But it would indicate that these forms may serve as a guide post pointing out the most probable location of the evolutionary path followed by the ancestors of *Synchytrium*.

Summary

Rhodochytrium does not appear in North Carolina until late in the spring; at first zoosporangia are most abundant, but late in the season only resting spores are found.

The cysts are independent, not connected through their rhizoids.

The zoospores are of the algal type and frequently contain starch grains, but are colorless except for the red anterior end; they are

active for half a day or more, but seem to conjugate rarely except when confined in small amounts of fluid.

The nature of the cyst (resting spore or zoosporangium) is determined on infection.

The red pigment which is found at all stages of the life cycle is haematochrome or an allied lipochrome.

Although the three races of *Rhodochytrium* appear to be geographically isolated and affect different hosts, no morphological differences were detected between them.

The germ tubes do not enter the stomata, but penetrate the epidermis at any point, usually in the vicinity of a vascular bundle.

The cysts, both resting and temporary, are uninucleate until full size is attained.

Their rhizoids extend along the vascular bundles, mostly in the phloem elements, which they destroy, but they also send haustoria to the vessels of the xylem.

When mature the resting spores have a two-layered cellulose exospore and a thick non-cellulose endospore; most of the reserve food is in the form of starch; the nuclei are considerably shriveled by the withdrawal of karyolymph.

The starch grains are similar to those commonly seen in the higher plants.

No plastids could be found, the starch grains appearing to be built up directly in the plasma.

The flaring necks of the zoosporangia are stopped by characteristic turbinate or bell-shaped plugs.

During the last mitoses there is a contraction which results in a pseudo-segmentation, but true segmentation appears to be brought about by the precipitation of membranes around the protospores.

There is a deeply staining body at the base of the cilia of the zoospores which is connected with the nucleus.

The primary nuclei, which reach the size of 50–60 μ, have enormous nucleoli and peculiar amorphous masses of chromatin like *Synchytrium decipiens*.

In the first type of mitosis, the spindle, which is usually unipolar at first, is formed from coarse acicular fibers that appear within

the nuclear cavity; it has no connection with the nuclear membrane. The spirem is formed from that part of the chromatin which lies in the equatorial region, the rest being cast out; it is frequently entirely within the spindle.

The second type of mitosis presents no unusual features.

No centrosomes or true asters were seen.

Amitosis is rare and abnormal in the zoosporangia.

Although superficially resembling *Eniophlyctis*, *Rhodochytrium* is not closely related to any known Archimycete.

But it appears to be closely related to the Protococcoideae through *Phyllobium*.

The Phyllobiae show considerable similarity to *Synchytrium* in gross morphology.

The cytology of *Rhodochytrium* bears a strong resemblance to that of *Synchytrium*.

These resemblances suggest that *Synchytrium* was derived from protococcoid ancestors.

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EXPLANATION OF PLATES XI–XVI

The figures were made with various combinations of Zeiss apochromatic and Spencer achromatic oil immersion lenses with compensating oculars. The magnification of the different figures is given in the description of each. The figures have been reduced one-third in reproduction, canceling the enlargement due to the camera and rendering them the same size as when seen in the microscope. All of the figures, except 16 and 19, were taken from the race of the parasite on *Ambrosia artemisiifolia*.

FIG. 1.—Young resting spores; ×334.

FIG. 2.—Somewhat older resting spore, spreading out irregularly in the tissue; ×334.

FIG. 3.—Cyst in which the basal portion has swollen out, although the protoplast has grown but little; rhizoids not in plane of section; nucleus with numerous spherules of chromatin connected by linin strands scattered through its cavity; ×334.

FIG. 4.—A full-sized resting spore whose wall is beginning to thicken, with that portion of the rhizoidal system which lay in the plane of section; ×334.

FIG. 5.—A portion of the rhizoidal system of a mature cyst, showing its relation to phloem and xylem; ×334.

FIG. 6.—Detail of a haustorium closely applied to a pitted vessel; from a cyst which had surrounded itself with a thick wall, hence the wall around the haustorium; ×334.

FIG. 7.—Two-layered resting spore, showing the shriveling of the nucleus and the cutting off of the rhizoids; ×334.

FIG. 8.—Mature three-layered resting spore; ×334.

FIG. 9.—Starch grains from mature cysts, showing variations in size and shape; ×2000.

FIG. 10.—Starch grains from a mature cyst under polarized light; ×2000.

FIG. 11.—Young zoosporangium with numerous fine strands of cytoplasm in which starch is forming; ×334.

FIG. 11*a*.—Detail from fig. 11, showing formation of starch grains; ×3000.

FIG. 12.—Young zoosporangium; ×334.

FIG. 13.—Young zoosporangium just beginning to swell out; ×334.

FIG. 14.—Young zoosporangium in the stem of the host; plug already developed, although the tubular form is still retained; ×334.

FIG. 15.—Full-sized zoosporangium, showing the characters of the primary cyst; ×334.

FIG. 16.—A typical turbinate plug from the race on *Asclepias pumila;* ×670.

FIGS. 17, 18.—Lamellate plugs; ×670.

FIG. 19.—An unusual form of lamellate plug from the race on *Spilanthes;* ×670.

FIG. 20.—A bell-shaped plug, apparently secondarily filled up; ×670.

FIG. 21.—A bell-shaped plug which is perforate; ×670.

FIG. 22.—A binucleate cyst with part of its rhizoids showing by a starch-free area the approximate size and position of the primary nucleus and the remains of the residual chromatin cast out during the primary mitosis; ×334.

FIG. 23.—The tetranucleate stage; drawn from two sections of a retort-shaped cyst with the bend perpendicular to the plane of section; the sporangium, and especially the vacuole, were therefore larger than is indicated in the drawing; ×334.

FIG. 24.—An 8-nucleate cyst in which the position of the primary nucleus is still clearly indicated by a starch-free area in the cytoplasm; only six nuclei in plane of section; ×334.

FIG. 25.—An oblique section of 16-nucleate cyst in which the nuclei were still bunched in the center; ×334.

FIG. 26.—A small cyst in the 32-nucleate stage; ×334.

FIG. 27.—A cyst with about 128 nuclei; ×334.

FIG. 28.—A segmented zoosporangium, the protospores still retaining the shape of the coenocyte from which they were derived; ×334.

FIG. 29.—A cyst apparently segmenting by cleavage furrows; ×1000.

FIG. 30.—Preliminary contraction resulting in pseudo-segmentation during the last mitoses; ×1000.

FIG. 31.—Segmentation by the precipitation of membranes in the cytoplasm: *a*, a wall just forming; *b*, a portion of cytoplasm left out between the segments; ×1000.

FIG. 32.—A newly formed protospore; ×2000.

FIG. 33.—A protospore rounded off and beginning to show the concentration of the chromatin; ×2000.

FIG. 34.—A protospore overstained, showing the body at the base of the cilia and its connection with the nucleus; ×2000.

FIG. 35.—A young spore with starch grains partly concentrated in the posterior end; chromatin concentrated into a single mass; ×2000.

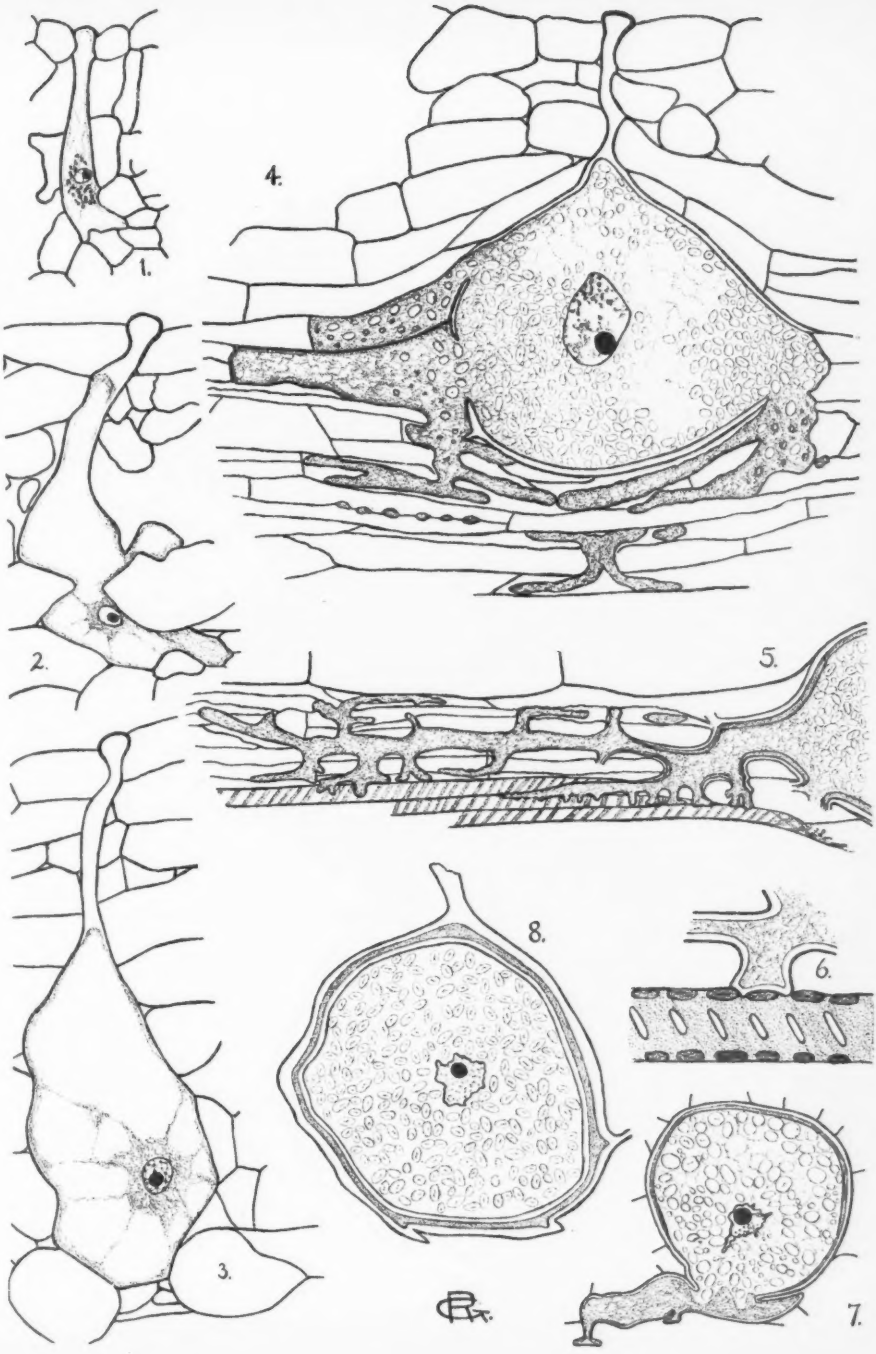
FIG. 36.—Mature spore from a section showing basal body and antero-posterior differentiation of the spore; ×2000.

FIG. 37.—Free swimming zoospore killed with osmic fumes stained with gentian-violet; ×1000.

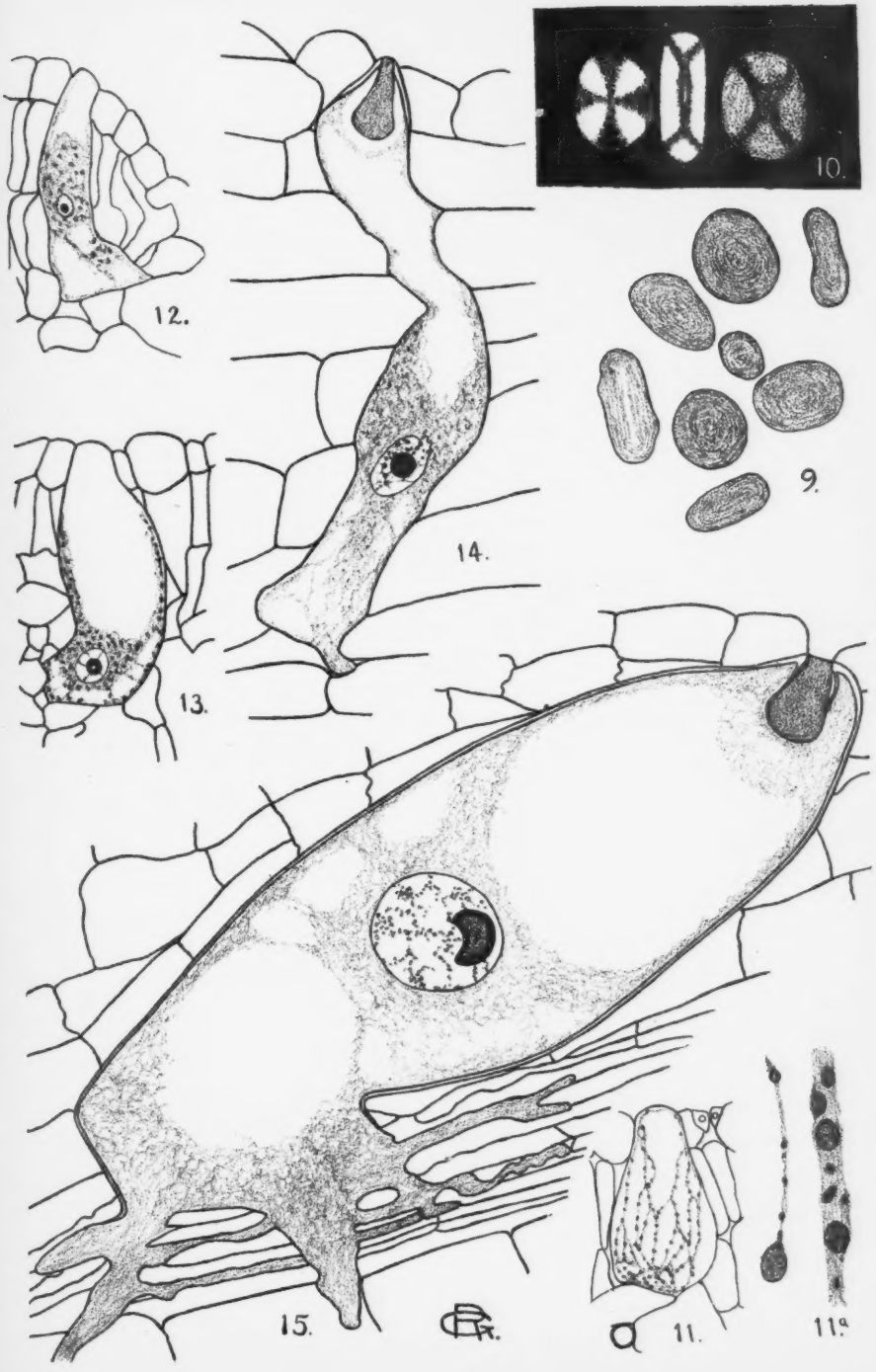
FIGS. 38–41.—Stages in the conjugation of the zoospores from living material; cilia diagrammatic; the difference in size between the gametes was accidental; there is no differentiation into microgametes and megagametes.

FIG. 42.—A nucleus from a very young cyst; ×2000.

FIG. 43.—A nucleus from a young resting spore; vacuolation of karyosome beginning; few connections between the chromatin spherules; ×2000.



GRIGGS on RHODOCHYTRIUM



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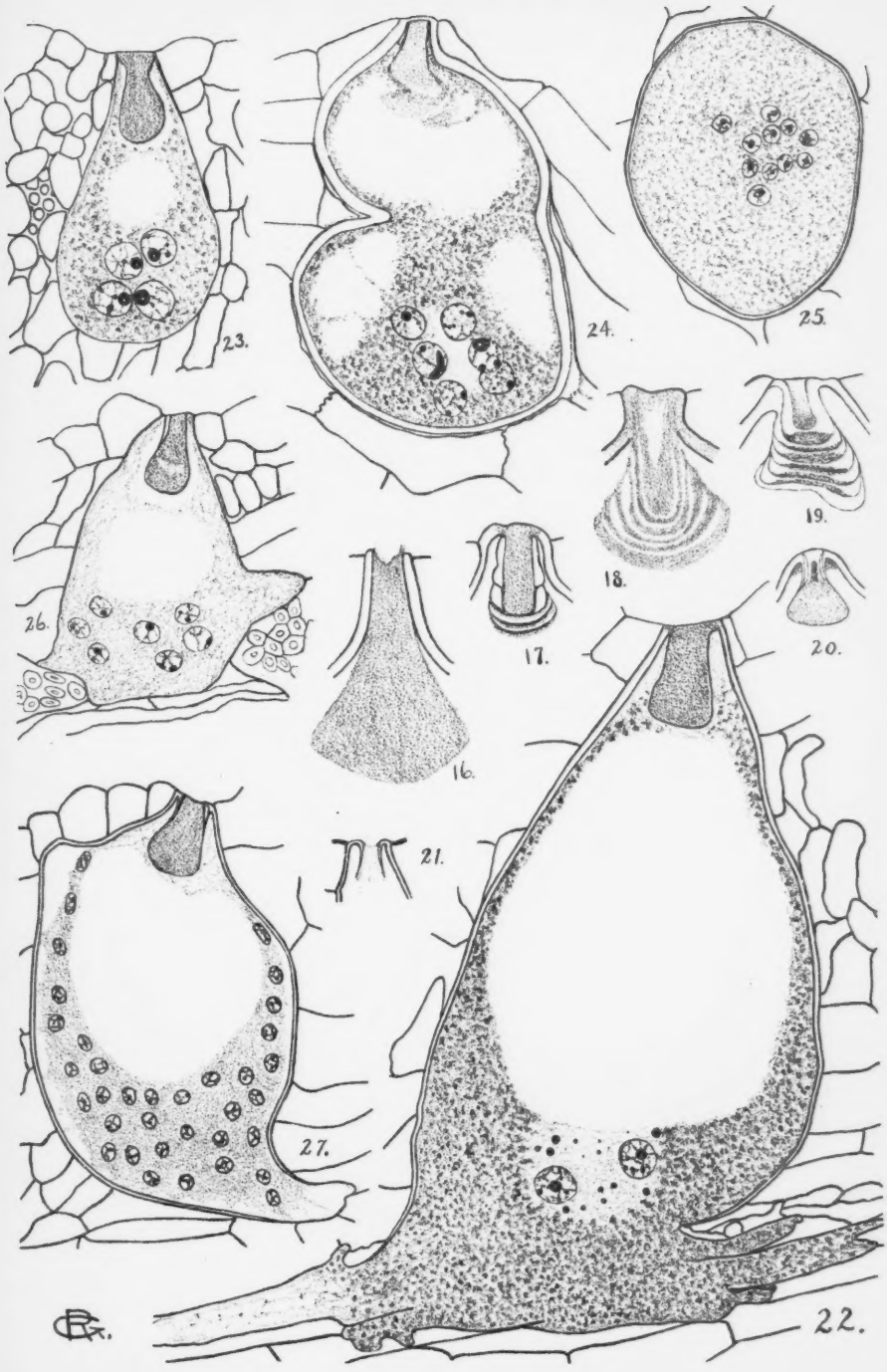

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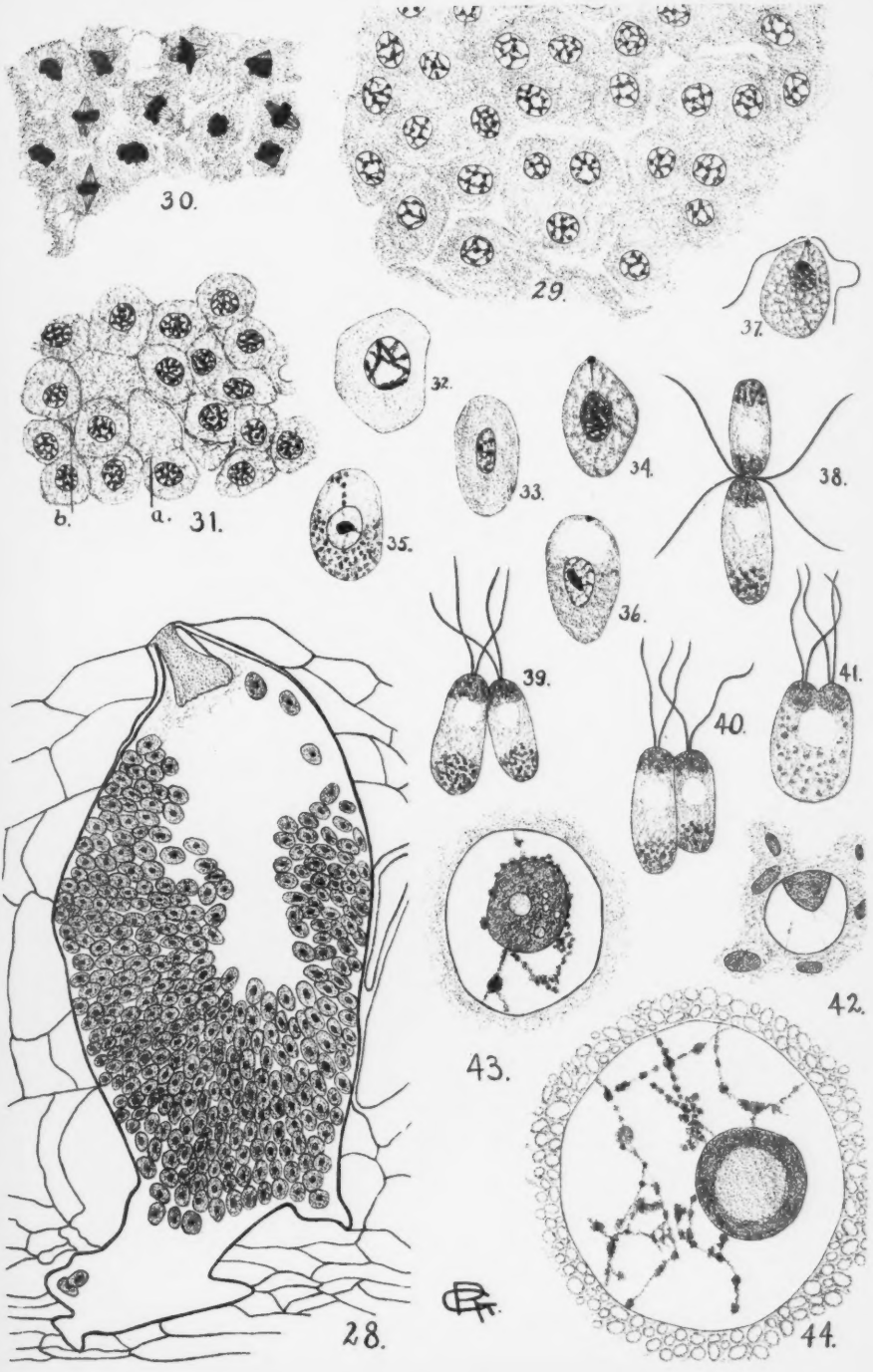

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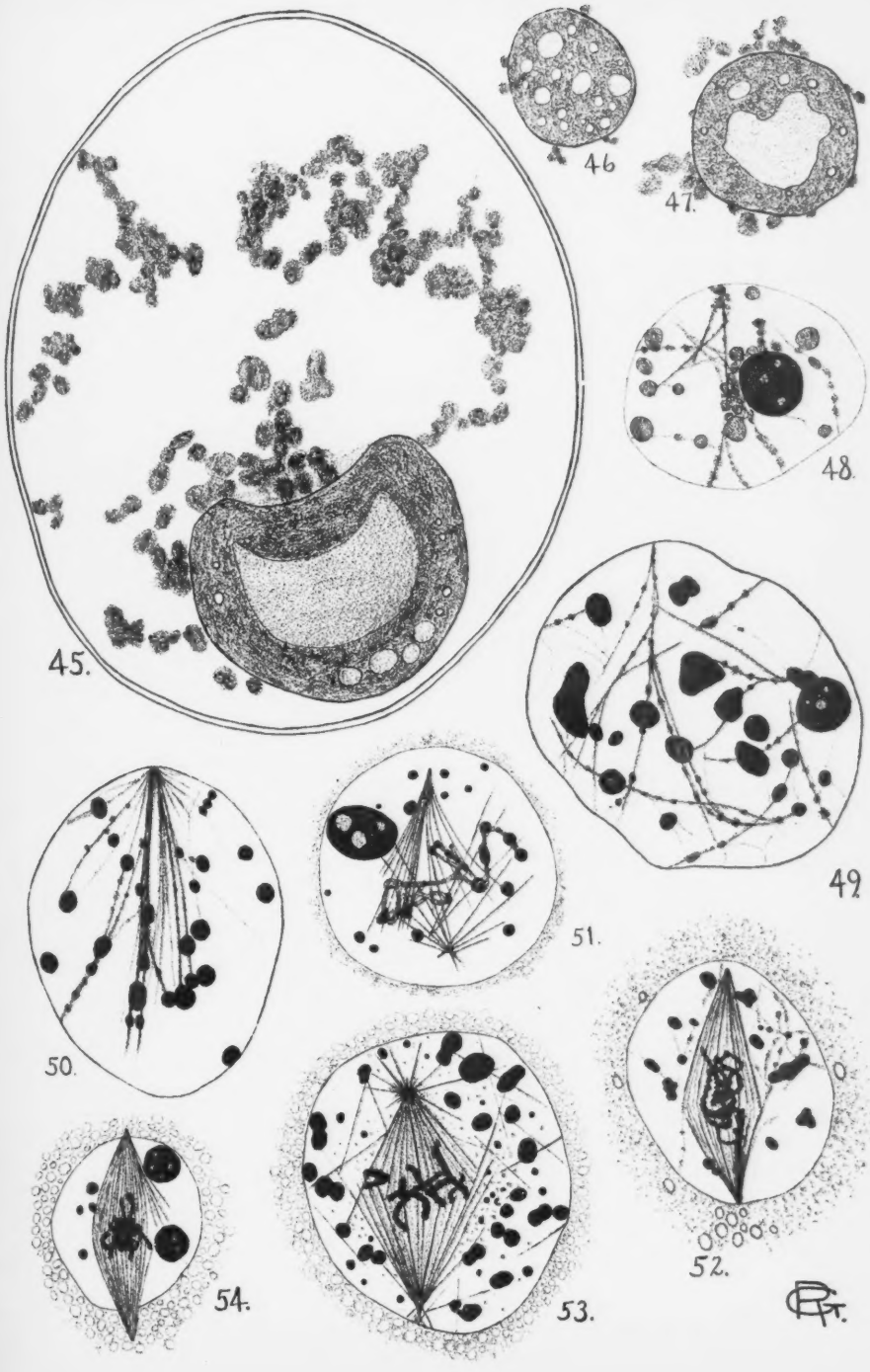

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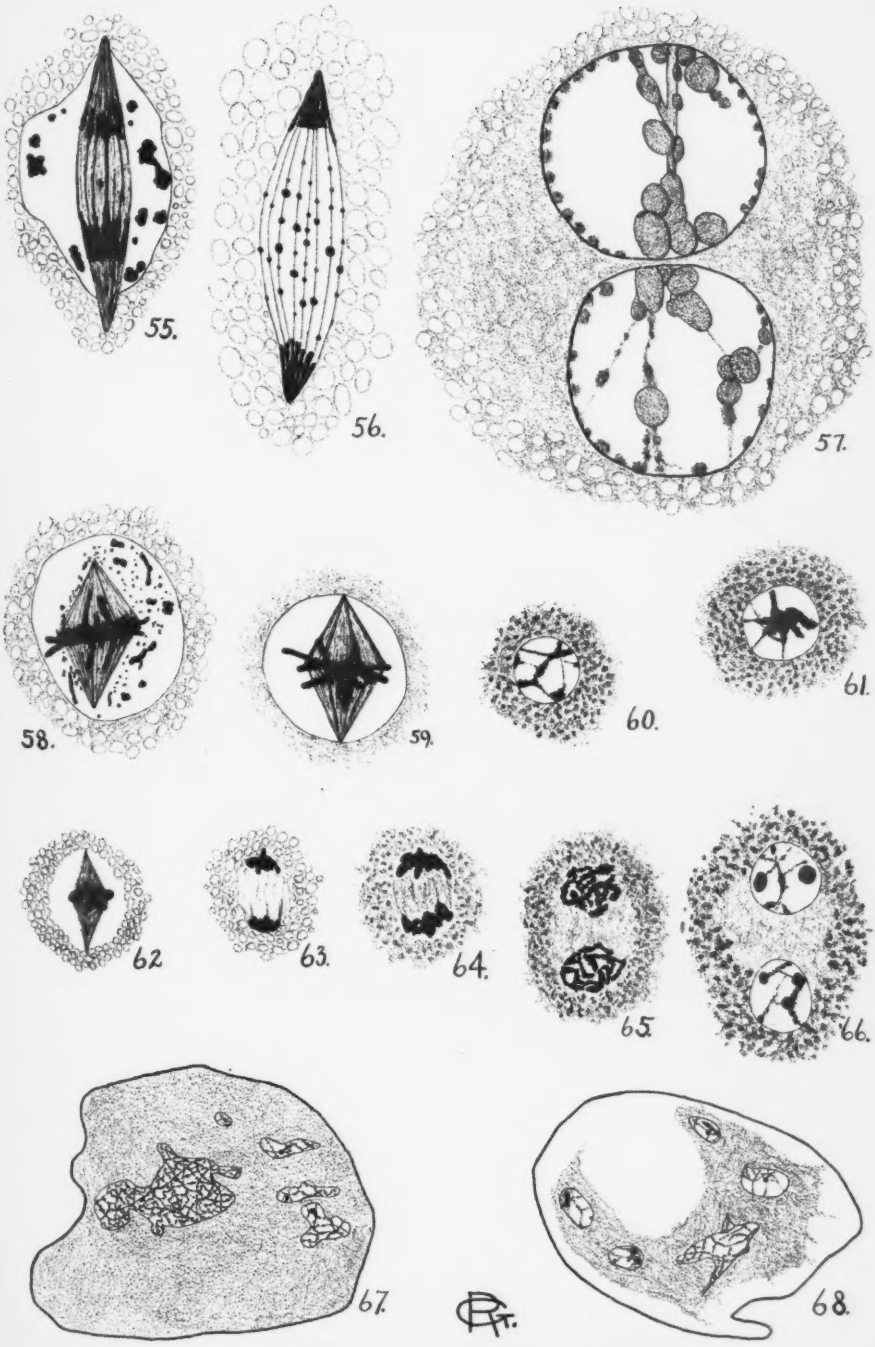

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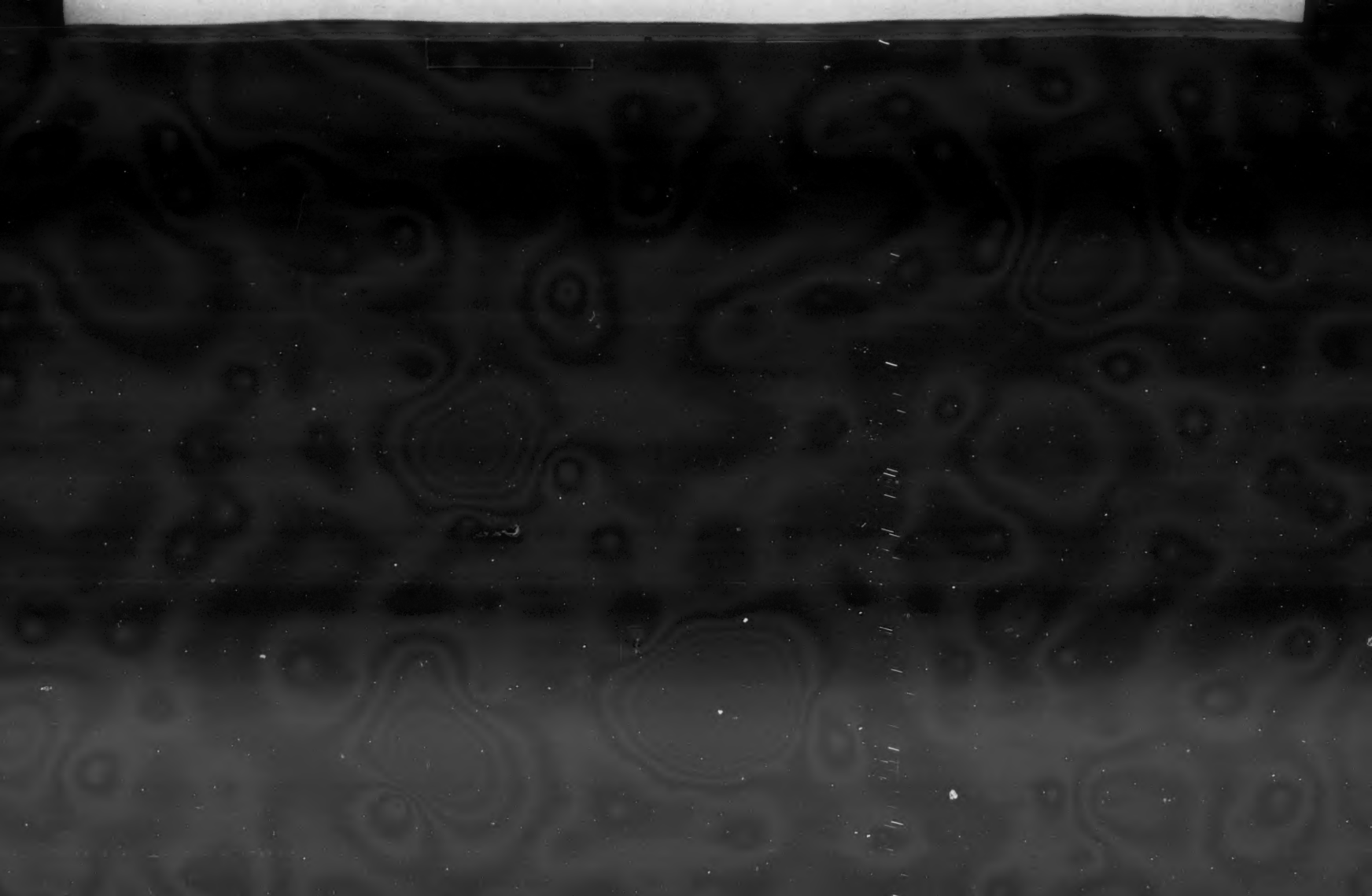

FIG. 44.—A nucleus from a half-grown zoosporangium; nucleolus with a single central vacuole; connections between the chromatin spherules unusually well developed; ×2000.

FIG. 45.—Enlarged drawing of the nucleus of fig. 15; ×2000.

FIG. 46.—A nucleolus with many small vacuoles; ×2000.

FIG. 47.—A nucleolus in which several small vacuoles have coalesced into a single central vacuole; ×2000.

FIG. 48.—The beginning of prophase from a tetranucleate cyst in which the other three nuclei were well advanced in mitosis; ×2000.

FIG. 49.—Early prophase in the primary nucleus; ×2000.

FIG. 50.—Later prophase in the primary nucleus; no indication of the opposite pole could be found; ×2000.

FIG. 51.—Prophase in a binucleate cyst, showing formation of the second pole of the spindle and of the spirem; ×2000.

FIG. 52.—Late prophase with spirem entirely within the spindle; third division; ×2000.

FIG. 53.—Metaphase in primary nucleus, showing chromosomes, masses of residual chromatin, and irregular disposition of fibers through nuclear cavity; aster at one pole largely accidental; ×2000.

FIG. 54.—Metaphase; spindle beginning to elongate, but spirem not yet completely segmented into chromosomes; third division; ×2000.

FIG. 55.—Anaphase, showing elongation of spindle and residual chromatin; fourth division; ×2000.

FIG. 56.—Early telophase; probably fifth or sixth division; ×2000.

FIG. 57.—Late telophase, showing persistence of outline of primary nucleus; first division; ×2000.

FIG. 58.—Late prophase in an intermediate nucleus; residual chromatin partly finely divided; ×2000.

FIG. 59.—Metaphase in similar nucleus; no residual chromatin; ×2000.

FIGS. 60, 61.—Resting nucleus and prophase of second type of mitosis from the same cyst; ×2000.

FIG. 62.—Metaphase, second type of mitosis; ×2000.

FIGS. 63–66.—Telophases, second type of mitosis; ×2000.

FIGS. 67, 68.—Cysts with irregular nuclei which are interpreted as the products of amitosis; ×334.

AMERICAN TRIASSIC NEOCALAMITES

EDWARD W. BERRY

(WITH PLATE XVII AND ONE FIGURE)

One of the most dogmatic statements of geology and paleontology refers to the almost complete change in the character of the floras in passing from the Paleozoic to the Mesozoic. This is a venerable dogma handed down from generation to generation until it has become almost axiomatic. Nevertheless, like most dogmas both scientific and otherwise, it was originally based upon lack of knowledge and its chief attribute is its unsoundness. A second misconception of a narrower kind is the current belief that the rocks of the Richmond coal-field in Virginia are of Keuper age. This latter seems to be based upon STUR'S comparison (**7**) with the Lunzer Lettenkohl flora of Austria, and upon the somewhat naïve reasoning of SHALER (**6**) that since the continued flora, whose affinities were early recognized, is unmistakably Rhaetic in its facies, it therefore is not of Rhaetic age, since it must have taken it untold years to spread over the world. This is an extreme application of HUXLEY'S principle of homotaxis, which is entirely unwarranted, and one which will be referred to again.

With increasing knowledge it has become obvious that one of the main reasons for the floral break at the close of the Paleozoic is conditioned by the unfavorable character of the early triassic sedimentation for preserving plant fossils. Among the forms which pass the magic boundary are *Glossopteris*, a probable pteridosperm, as WHITE (**9**) and others have pointed out; *Yuccites*, *Noeggerathiopsis*, *Krannera*, *Eolirion*, and *Cardiocarpon*, and possibly other more or less indefinite fossils may represent the Cordaitales in the older Mesozoic, as ZEILLER (**12**) has recently suggested. *Sigillaria* is represented by the form which was christened *Pleuromeia* by CORDA.

A number of genera of cycadophytes are already differentiated in the late Paleozoic, and it has long been evident that, in spite of the contrary tradition, the triassic Equisetales are more like their paleozoic than their existing representatives.

HALLE (**3**) has recently suggested setting aside certain species formerly referred to the illy understood equisetaceous genus *Schizoneura*, to constitute a separate and perhaps collaterally related genus, for which he has proposed the name *Neocalamites*, and which he compares with the paleozoic *Calamites*. WILLS (**11**) has supplemented this suggestion by comparing the species which are left in the genus *Schizoneura* with GRAND'-EURY'S *Calamodendron* type of paleozoic *Calamites*, and LIGNIER (**5**) has recently described *Calamitomyelon Morierei* from the French Lias.

The previously known species of *Neocalamites* are three in number: *N. meriani* (Brongn.), *N. hoerensis* (Schimper), and *N. carrerei* (Zeiller), and all are Keuper or Rhaetic in age. Both this genus and *Schizoneura* have been discussed by WILLS (**10, 11**) since the appearance of HALLE'S paper, so that further comments are unnecessary.

In an examination of the recently reopened Carbon Hill mine in the Richmond coal-field of Virginia, two equisetaceous types were discovered which are apparently referable to *Neocalamites*. The one, represented by very abundant but exceedingly poor remains, is identified with *Schizoneura virginiensis* described from this area in 1883 by FONTAINE (**2**). This represents a species which appears to be very close to *Schizoneura meriani* Brongn., and consequently referable to *Neocalamites* as defined by HALLE. It is described by FONTAINE as having several very fine veins, but this character is very obscure in all of the material and may or may not be true. It is something more than a coincidence that a like state of affairs seems to prevail in *S. meriani* described ordinarily as uninerved, but which WILLS has found to sometimes show several fine median veins. The other is an entirely new and remarkable type, which, in its superficial features at least, is very suggestive of the paleozoic *Calamites* with the *Annularia* type of foliage. The two were not found associated, although according to the mine engineer they both came from the same level, that is, the roofing shales of the 6-foot seam. The specimens were collected from the dumps, and their contemporaneous growth should therefore be accepted with caution, since the facies of the plants associated with each is slightly different, but probably equally explicable

either by a slightly different environment or by their having come from a somewhat different level. The dumps from which collections were made represent two openings: the eastermost of which is a slope mine following the dip of the 6-foot seam which comes rather close to the surface near the entrance; the westermost, 200 yards away, known as the Engine Hill mine, is a shaft which was said to strike the same 6-foot seam at a depth of 250 feet, but which was not being worked at the time of the writer's visit. *Neocalamites virginiensis* (Fontaine), as it should be called, was collected from the eastern dump, where it was associated with vast numbers of fronds of *Macrotaeniopteris magnifolia* (Rogers) Schimper, and with the equally abundant stem remains of large and small specimens of *Equisetum* and very rare fern fragments, the whole constituting a typical triassic swamp assemblage.

In the flora associated with the new species of *Neocalamites* the remains of *Equisetum* were almost entirely absent, *Macrotaeniopteris* was not seen, and ferns and cycadophytes greatly predominated. The pinnules of the enormous *Sphenozamites Rogersianus* Fontaine were often packed together in solid masses, among which some nearly complete fronds were collected. *Clathropteris* was common and some of the specimens were remarkably complete. The fern genera identified by FONTAINE as *Acrostichides*, *Mertensides*, etc., were abundant, and various *Ctenis*-like and *Pterophyllum* forms were collected. Sparingly represented were those curious forms described by EMMONS (**1**) over 50 years ago from the North Carolina Triassic area under the name *Lepacyclotes*.

EMMONS (**1**) described two species in 1856 as *Lepacyclotes ellipticus* and *L. circularis*. These were discussed by FONTAINE (**2**) in 1883 in his monograph of the Virginia Triassic. At that time he considered them as probably representing a single species of crushed cone closely allied to *Araucaria*, and they were renamed by him *Araucarites carolinensis*. In returning to the same subject in 1900, after the rediscovery of the EMMONS' collection, he abandons this view and returns to EMMONS' names, his final opinion being that the disklike forms represent *Equisetum* diaphragms, and the scalelike forms fragments of *Equisetum* stems (**8**). I am not in a position to discuss the first assumption, since I have not seen the

material. The second is clearly erroneous. The Virginia specimens are cone scales, and while it is merely a supposition, I would be inclined to consider them as representing contemporaneous cycadophytes rather than Araucarieae.

The new species of *Neocalamites* was collected by the writer and T. E. WILLARD of the U.S. National Museum, and is named in honor of Dr. F. H. KNOWLTON, who was instrumental in bringing about the writer's visit to the mine.

Neocalamites Knowltoni, sp. nov.

The main axis is preserved for a distance of 14 cm., and shows 8 nodes in this interval. It is slender, being 8 mm. across the flattened proximal end, and 6 mm. across the flattened distal end. The nodes are about 2 mm. apart, and show no traces of leaves or sheaths. The surface is lined longitudinally, and there is no apparent alternation of vascular strands at the nodes, a variable feature in this whole class of plants and much less important than was formerly supposed to be the case. Leaf-bearing branches opposite. They were possibly in whorls in some cases, since there appear to be 1 or 2 branch scars just above certain of the nodes, which, if they indicate fully developed branches in addition to the two opposite ones which are preserved, would make the leaf-bearing branches 3 or 4 in number at these respective nodes. It is believed, however, that the functional branches were usually but 2 in number and opposite, since no traces of additional branches are preserved. If other branches developed occasionally, they may be regarded as reversions to an ancestral verticillate arrangement, and the branch scars above mentioned may be interpreted as the scars of such aborted or non-persistent branches.

Lateral foliage-bearing branches preserved for a length up to 7 cm., slender, being not over 3 mm. across at the proximal end after flattening due to the compression of fossilization. Internodes short, about 1 cm. in length, longitudinally striated.

Leaves in whorls of 9 or 10 at the nodes of the lateral branches, apparently free, although they may be slightly united at the base as in the paleozoic Annularias, the material collected being not entirely conclusive on this point. They are linear-lanceolate in

outline, with an obtusely pointed apex, about 1 cm. in length by 1.5 mm. in greatest width. All the leaves in a whorl are of approximately the same size.

From their position as fossilized, they seem to have been superimposed from node to node, and each verticil seems to have been in a plane very oblique to the supporting axis and not at right angles to it, so that the foliage-bearing branch with its unit whorls is, as a whole, bifacial.

Leaf substance thick and coriaceous. Within the limits of the specimen there is scarcely any diminution in the size of the leaves or length of the internodes distad from the main axis, although the branch itself tapers slightly.

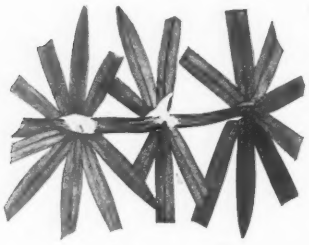

FIG. 1

The venation is puzzling because of the thick nature of the leaves and their indifferent preservation, some leaves apparently showing a thick prominent midrib, while in others its place was apparently occupied by what seem to be several very fine vascular strands.

The writer's final conclusion is that each leaf has a single midrib, which was broad, but immersed in the leaf substance and not at all prominent in life. This midrib may have been made up of several vascular strands, and varying conditions of preservation account for the deceptive appearances in some of the leaves.

The accompanying text figure (fig. 1) is from a drawing (×4) which shows three verticils, and is drawn from a counterpart of the type which is shown natural size on pl. XVII.

In the absence of any very complete knowledge of the older mesozoic Equisetales, the affinities of the present species are more or less conjectural. It fulfils all of the requirements of HALLE's definition of the genus *Neocalamites*, and the genus itself seems to be a natural one. It is more like *Calamites*, however, than the species which HALLE has referred to the genus, and suggests most strongly the *Annularia* type of paleozoic calamite foliage, as, for example, the widespread type known as *Annularia sphenophylloides*, the only difference being that in the triassic *Neocalamites* the leaves

of a whorl are not dissimilar in size. A second possible difference is that the leaves appear free to the base. This is not positively ascertained, however, and is of slight importance at best, since there must have been a progressive change from free leaves to united sheaths and vice versa, when the group as a whole is considered, and the two lines of variation may have been contemporaneous within the phylum.

There is also a suggestive resemblance between the present species and the forms from the homotaxial Rhaetic deposits of Tonkin described by ZEILLER (**13,** p. 132. *pl. 35. figs. 2–7*) as *Annulariopsis inopinata*, gen. et sp. nov. This remarkable form, while based upon rather incomplete material, shows whorls of 16–24 lanceolate-spatulate, uninerved, free leaves, the main difference betwen it and *Neocalamites Knowltoni* being the uniform size of the leaves of the latter. In *Annulariopsis* each whorl shows short leaves on one side and long leaves on the opposite side, with a regular gradation between the two, the maximum being 100 per cent larger than the minimum.

It appears, therefore, that as regards habit and superficial characters *Neocalamites* was closely allied to and undoubtedly descended from some paleozoic *Calamite*. On the other hand, it does not seem to be genetically related to *Schizoneura*, although it comes after it in time.

Neocalamites Knowltoni was a large plant, and it is quite possible that some of the fragments of large stems 10 or 12 cm. in diameter, which are so abundant at some horizons in the coal-field, may represent the main axis. The axis of the specimen, with its leaf-bearing subordinate branches, is interpreted as a lateral branch which was distinctly bifacial in habit. The material from the Triassic is too limited for certainty on this point, but it seems difficult to account for the uniform orientation of the numerous whorls of leaves on the distichous branches by appealing to compression during fossilization, which it would seem reasonable to suppose on even a single specimen would flatten some leaves in one direction and some in another and would break off or bend some of the leaves.

The obliquity of the plane of the verticils in *Annularia* is often insisted upon in the diagnosis of this paleozoic type, although some

authors explain this feature by compression during fossilization. In this case also the mechanical orientation of the *Annularia* whorls in the thousands of specimens which have been collected is difficult if not impossible of adequate explanation if the theory that the leaves in life radiated at right angles to the axis be adopted. The present specimen comes from the immediate vicinity of the old Carbon Hill mine, about one mile south of Gayton on Tuckahoe Creek, near the western border of Henrico County, Virginia, from beds of undoubted Rhaetic age, and the type is deposited in the U.S. National Museum, duplicate and less perfect material being retained in the collections of the Johns Hopkins University.

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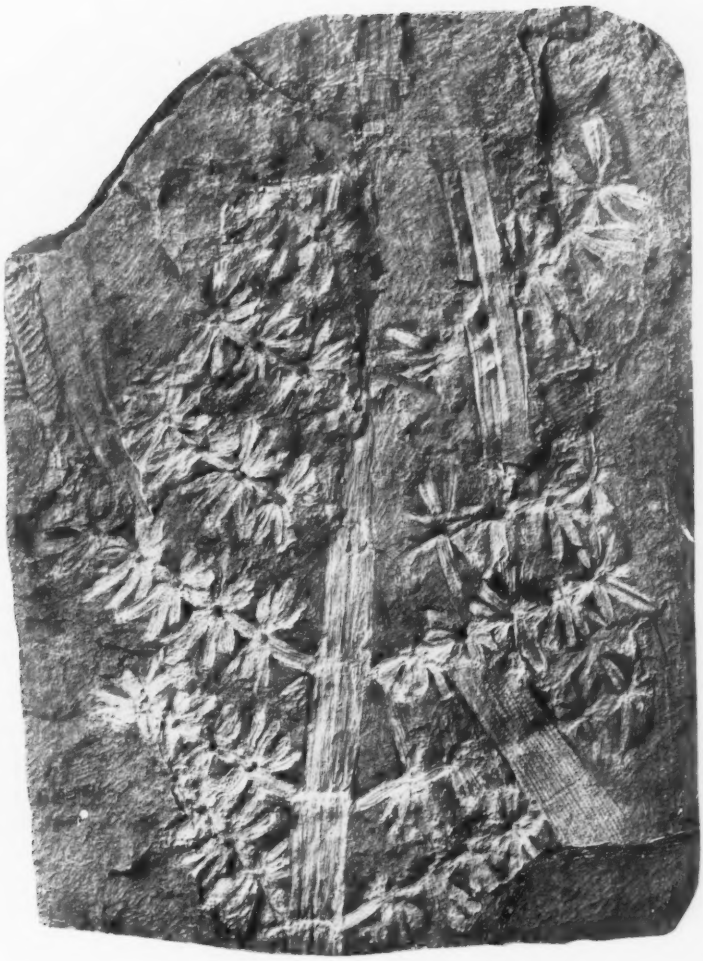
12. ZEILLER, RENÉ, Les progrès de la paléobotanique de l'ère des gymnospermes. Progressus Rei Botanicae **2**:171–226. 1907.
13. ZEILLER, R., Fl. foss. des gites de charbon du Tonkin. Paris. 1903.



Neocalamites Knowltoni Berry

CURRENT LITERATURE

BOOK REVIEWS

Vegetation der Erde

XIII. NORTH AMERICA

It is unfortunate that ENGLER and DRUDE should have decided to devote but one volume of the *Vegetation der Erde* to North America. Such a decision seems out of harmony with the rest of the work. It might have been expected that a work published in Europe would devote separate volumes to such relatively limited areas as the Carpathians, the Caucasus, the Balkan countries, and the North German heath. However, a somewhat comparable plan has been followed with regard to Africa, three volumes having already been issued, with more promised. Even in South America, a volume has been devoted to Chile and another to the Peruvian Andes. With such a plan, it is a fundamental mistake to devote but one volume to North America.[1] HARSHBERGER prepared himself as well as he could for the impossible task he was asked to undertake by years of study and by trips to all the more important phytogeographical areas of North America except Central America and the arctic region. Under the circumstances, the volume is one with which the author may be fairly well satisfied. It is a veritable mine of information, in which there may be found the chief results of the phytogeographic work accomplished upon our continent. There are many errors of detail throughout the volume, errors both of omission and of commission, and some are rather serious. To many, and especially to taxonomic specialists of local areas, these errors will loom large. To those of broader viewpoint, however, the numerous errors will be subordinate to the relatively successful completion of one of the most stupendous tasks ever undertaken by a single botanist. HARSHBERGER deserves and will receive the gratitude of all future plant geographers in our country, for he has vastly lightened their labors. They will value this work because of its helpfulness as a guide to literature, and because of its broad comparisons and generalizations; it will be for them an easy matter to correct the errors of determination or of synonymy and the mistakes in spelling that seem such grevious matters to some of the reviewers. This volume is the first of the series to appear in a tongue other than the German. It is a pleasure to congratulate ENGLER and DRUDE for their broad-minded conception in this

[1] ENGLER, A., and DRUDE, O., Die Vegetation der Erde. XIII. HARSHBERGER, J. W., Phytogeographic survey of North America. pp. lxiii+790. *map. pls. 18. figs. 32.* Leipzig: Wilhelm Engelmann (also G. E. Stechert & Co. New York). 1911. *M.* 52 (subscription price *M.* 40).

matter, thus adopting a plan which ENGLER had previously adopted in the *Pflanzenreich*.

Following the preface is a German summary of the contents of the volume by DRUDE. Part I gives a survey of floristic and phytogeographic work in North America, and also a most useful bibliography. Part II contains an account of the geography and climate of the continent, together with some plant statistics. Part III has to do with the geologic evolution of the North American flora from the Cretaceous to the present. Here there are discussed the sudden appearance of Cretaceous angiosperms, and the influence of Pleistocene glaciation in the destruction of species and in the production of relict endemism. A detailed account is given of the postglacial history of our flora. To the north there has been a succession of forest types, culminating in the dicotyl forests which now generally dominate. Interesting accounts are given of recent changes in the vegetation of the coastal plain. HARSHBERGER aligns himself with those who regard the prairies as sufficiently explained by taking account of historical factors. This part closes with a description of the affinities of the North American flora and a list of phytogeographic classifications pertaining to North America.

Part IV, which comprises more than half of the volume, presents in some detail the phytogeographic regions of North America, and is accompanied by a colored map which makes it easy to follow the text. There are seven chapters, dividing the continent into as many "zones": (1) The arctic and subarctic zones; the latter is subdivided into the Labrador, Hudson Bay-Keewatin, Mackenzie, and Alaska districts. (2) The North American temperate zone, Atlantic section; subdivided into the St. Lawrence–Great Lake, Atlantic–Gulf Coast, and Piedmont-Appalachian-Ozark regions. Among the districts most fully treated are New Brunswick (based largely on GANONG's studies), the New England mountains, the Adirondacks, the New Jersey pine barrens, the coastal formations, and the various forest districts. (3) The North American temperate zone, interior xerophytic sections; subdivided into the prairie, Rocky Mountain, and Great Basin regions. (4) The North American temperate zone, Pacific section; subdivided into the Sitkan, Columbian, and Californian regions. (5) The Mexican subtropic zone and mountain regions. (6) The North American tropic zone, Mexican and Central American section. (7) The North American tropic zone, West Indian section. The illustrations are good, but are much too few to depict properly the vegetation of a continent.—HENRY C. COWLES.

NOTES FOR STUDENTS

Cecidology.—The similarity of plant galls and animal tumors is attracting the attention of workers in various parts of the world. SAUL[2] has issued a preliminary paper in which he expresses the opinion that some of the various

[2] SAUL, E., Beziehungen der Acari zur Geschwulstatiologie. Centrabl. f. Bakt., Paras., und Infekt. **59**:400–406. 1911.

cancerous growths of animals may be due to insects, and that improved technique will throw light upon the problem. He expresses regret that the progress of this line of work has been so slow, and reviews some of BEIJERINCK's works which he believes have the most important bearing on the subject. He believes that the insect (larva) secretes an enzyme which causes a proliferation of the body cells without changing their physiological function, and that this enzyme can be transferred from cell to cell. The possibility of insects being the cause of such growths in animals was taken up in Krebs Institute in Heidelberg in connection with the study of an endemic disease of rats. The rats suffered from an infectious disease causing papillose tumors, but the technique was not satisfactory in demonstrating the exact cause. The author presents a number of microphotographs of insects and sections of plant galls, which he discusses in relation to their similarity to animal tumors. He also briefly reviews the works of several authors who have expressed similar opinions.

One of the most valuable discussions concerning the character and grouping of galls is by KÜSTER,[3] who suggests a division of the galls into two groups, "histoide" and "organoide." The former includes such simple structures as cork formed about wounds, and the more complex structures such as oak galls, which, although made up of plant tissue, are unlike any of the plant organs. The latter includes the formation of roots from a leaf, those modifications of stems and leaves which are usually known as teratological structures, and those modifications of parts which are due to fungi and insects. The author discusses the modification of the leaves of the willow due to *Aphis amenticola*, the witches' brooms of the cherry, birch, and fir trees due to *Exoascus cerasi*, *E. betulinus*, and *Melampsorella caryophyllacearum*, the cone gall of *Salix caprea* which is due to *Rhabdophaga rosaria*, the leaf modifications of *Populus tremula* which are due to *Eriophyes dispar*, the formation of new roots on *Poa silvestris* due to *Cecidomyia poa*, the formation of adventitious parts on *Fraxinus ornus* and *Pteris quadriaurita* due to *Eriophyes fraxini* and *Taphrina laurencia*, as illustrating the various types of organoides. This is followed by a brief discussion of a number of insect and fungus galls which possess characters of both histoides and organoides. The author attaches no importance whatever to the cause, but bases his classification entirely on the character of the deformity without regard to the fungus or insect which produces the stimuli.

A brief but very interesting paper by HARRIS[4] shows that as vague a subject as teratology may present problems for serious investigation. His studies indicate that the anomalous fruits of *Ptelea trifoliata* are rare, the greatest variant being less than 1 per cent. Although the author states that he considers the number of pods studied entirely too small for satisfactory

[3] KÜSTER, E., Ueber organoide Misbildungen auf Pflanzen. Aus der Natur. 7:673–685. 1911.

[4] HARRIS, T. ARTHUR, Teratological fruits of *Ptelea*. Bull. Torr. Bot. Club 38:385–387. 1911.

conclusions, yet his paper demonstrates one of the many possibilities in this branch of cecidology.

Among the more important taxonomic papers is LEEUWEN-REIJNVAAN'S[5] fifth contribution on the galls of Java. They describe 49 different galls, 21 of which are figured. These galls are grouped with reference to the plants on which they occur, and are assigned to genera but not given specific names.

MASSALONGO[6] describes 8 new species of galls, 7 of which are due to insects and 1 to fungi.—MEL T. COOK.

Sand dune and subalpine vegetation in New Zealand.—With a sand dune area of some 300,000 acres, the question of its reclamation becomes one of national importance in New Zealand, especially since through their advance the dunes ruin much valuable land. In a previous paper by COCKAYNE, reviewed in this journal,[7] the ecological problems of these areas were discussed, and the influence of pasturing, tree cutting, and burning was noted as increasing the movement of the dunes to a marked degree. In a more recent publication,[8] the same investigator has restated many of his former conclusions, and in addition has discussed the best methods for reclaiming actively moving dunes, for protecting farm lands and other valuable areas from the encroaching sand, and for preventing the rejuvenescence of fixed dunes. The efficiency of marram grass (*Ammophila arenaria*) as a sand holder is emphasized, while the tree lupin (*Lupinus arboreus*) is found to be an excellent shrub to reinforce the grass and to act as a pioneer in the process of reforestation, which is recognized as offering the greatest permanent stability combined with the transformation of a barren and dangerous into a productive region. Some action by the New Zealand Government seems likely to result from these recommendations.

COCKAYNE and his associates have also been making a preliminary ecological survey of a mountainous area in the Southern Alps region of New Zealand.[9] The highest peak here is Mt. Arrowsmith, 9171 ft., and it is surrounded by others of somewhat less altitude. Glaciers occur rather plentifully, and the region gives evidence of much more extensive ice sheets in the past. Two climatic regions are here closely adjacent, due to differences in rainfall depend-

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[5] LEEUWEN-REIJNVAAN, J. und W. DOCTERS VAN, Einige Gallen aus Java. V. Marcellia **10**:65–91. 1911.

[6] MASSALONGO, C., Zoocecidii e fitocecidii rare o nuovi. Marcellia **10**:94–97. 1911.

[7] BOT. GAZ. **50**:478. 1910.

[8] COCKAYNE, L., Report on the dune areas of New Zealand, their geology, botany, and reclamation. Department of Lands. 4to. pp. 76. *pls. 72*. 1911. Wellington: John Mackay, Government Printer.

[9] SPEIGHT, R., COCKAYNE, L., and LAING, R. M., The Mount Arrowsmith district; a study in physiography and plant ecology. Trans. N.Z. Institute **43**:315–378. 1911.

ing upon exposure to the moist westerly winds. In the more mesophytic region, with an annual rainfall of about 250 cm., a rain forest formation develops, its conspicuous trees being *Podocarpus Hallii*, *Librocedrus Bidwillii*, and two species of *Phyllocladus*.

In the more xerophytic portion of the region, while the precipitation is quite considerable, exposure to strong insolation and almost continuous winds produces a steppe formation. This is developed upon several sorts of rocky substrata, and is characterized by an abundance of shrubby, tussock, and cushion plant forms. The succession in some stony river beds has been more carefully studied,[10] and may indicate the interesting results likely to follow more detailed investigation of other similar situations. From a pioneer *Epilobium* association there develops one characterized by the genus *Raoulia*, plants remarkable for the development of strikingly unique cushion forms. Various species of these curious composites are abundant throughout the steppe formation, reaching their most striking development in the "vegetable sheep" association of certain rocky hillsides. The climax association of the river beds is a subalpine scrub of the usual type.—GEO. D. FULLER.

Iron bacteria.—A recent contribution by LIESKE[11] is of importance in that it supplements our knoweldge of the iron bacteria that has come to us largely through the writings of MOLISCH. It also revives interest in WINOGRADSKY'S theory of iron assimilation, and illustrates in its comparative results the ever-present danger of generalizing from a too narrow inquiry into the field of research.

The author has made an intensive study of one of the numerous species of iron bacteria known as *Spirophyllum ferrugineum* Ellis, enlightening us regarding its specific cultural and physiological peculiarities. Unlike *Leptothrix ochracea* studied by MOLISCH, this bacterium does not grow in a medium containing organic matter; neither in an iron-free medium, nor in a medium containing iron salts other than ferrous carbonate or bicarbonate, nor salts of any of the other metals.

Of chief physiological importance is the experimental proof that the organism can utilize the carbon of CO_2 introduced into a flask from which every other source of carbon can be excluded; the nutrient medium contains in solution inorganic salts, iron filings are added, and CO_2 is furnished to the extent of 1 per cent of the air in the flask. Naturally, then, issue is taken with MOLISCH, who in his recent monograph (*Die Eisenbakterien*[12]) denies the correctness of WINOGRADSKY'S hypothesis that iron bacteria require iron

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[10] COCKAYNE, L., On the peopling by plants of the subalpine river bed of the Rakaia. Trans. and Proc. Bot. Soc. Edinburgh **24**[3]:104–125. 1911.

[11] LIESKE, R., Beiträge zur Kenntnis der Physiologie von *Spirophyllum ferrugineum* Ellis, einem typischen Eisenbakterium. Jahrb. Wiss. Bot. **49**:91–127. 1911.

[12] Reviewed in this journal, **50**:464.

for a source of energy and nutrition. The results of this investigation on *Spirophyllum ferrugineum* would make it appear that for certain of the iron bacteria the storage of iron is not brought about by mechanical means, as MOLISCH suggests.

Using PFEFFER'S phrase "chemosynthesis," LIESKE draws attention to the fact that CO_2 is chemosynthetically assimilated by certain other species of oxidizing bacteria, such as WINOGRADSKY'S nitrate and nitrite bacteria: those capable of transforming thiosulphates into tetrathionic and sulphuric acids; those splitting up H_2O_2; or those that are able to oxidize CH_4 and CO and utilizing the carbon contained therein directly. He then is of the opinion that this particular iron bacterium, *Spirophyllum ferrugineum*, acts in an entirely similar way, inasmuch as from an elementary analysis of organisms grown in a fluid medium containing inorganic salts, metallic iron, and no other source of C other than that supplied indirectly by the action of CO_2 on the metallic iron (forming ferrous carbonate), he was able to prove a distinct increase in the C content of the mass of bacterial filaments. LIESKE calculates that the quantity of ferric oxide that *Spirophyllum ferrugineum* must form from ferrous carbonate to gain one part of C is 750 parts, if roughly estimated in parallel to the requirements of the nitrite bacteria.

That this use of ferrous carbonate for the sole purpose of chemosynthesis of C applies to *all* iron bacteria is, as LIESKE states, questionable; but the fact that it now seems proved in the case of this particular iron bacterium lends new life to the hypothesis of WINOGRADSKY; and at the same time makes it necessary that new and more widespread study of the several species of iron bacteria be undertaken in a most thorough manner.—NORMAN MACL. HARRIS.

Forests of the Philippines.—A rather complete discussion of the economic aspect of the forests of the Philippines, based upon the investigations of WHITFORD, has been issued as a bulletin of the Bureau of Forestry of these Islands.[13] The first part deals with the classification of the various forest types, the importance of the diptocarp types being emphasized, the amount and quality of the lumber, the uses to which it is adapted, the character of the lumbering operations, and the forest products other than lumber. It includes the results of mechanical tests of 34 Philippine woods and a bibliography of both Spanish and English publications on the forestry of the islands. The second part is devoted to the description and illustration of over 100 of the principal tree species. The descriptions relate principally to the trunk, branch, leaf, and wood characters, and not to those of the reproductive parts.

[13] WHITFORD, H. N., The forests of the Philippines. P.I. Department of the Interior, Bureau of Forestry. Bull. no. 10. Part I, Forest types and products. pp. 94. *pls. 27*. Part II, The principal forest trees. pp. 113. *pls. 103*. Manila: Bureau of Printing. 1911.

The plates include photographic studies of the trunks and drawings of the leaves and fruit.

The forests dominated by members of the Dipterocarpaceae are by far the most important both in extent and in volume of merchantable timber. The composition of these forests is a simple one from the forester's or lumberman's standpoint, a given area seldom having more than 15 or 20 species of economic importance; and in the most productive of the dipterocarp forests, known as the lauan type, 95 per cent of the timber belongs to 6 dominant species. The same forest is complex from the standpoint of the botanist, since it contains 150–200 tree species, the greater number being too small to be economically important. Once within the tangled mass of lianas about the openings, these forests are easy to penetrate.

In addition to an abundance of timber for general construction purposes, these forests produce excellent substitutes for mahogany and lignum vitae, many valuable furniture woods, and woods suitable for carving, engraving, and numerous other purposes. Among the other forest products are resins, oils, rubber, rattan, and bamboo. Lumbering methods have been largely primitive, but these are being replaced by more scientific ones, which promise to produce not only all the timber required for use upon the islands, but considerable quantities for export.—GEO. D. FULLER.

African sand dunes.—The vegetation of a narrow border of sand dunes along the shores of the Bay of Algiers has been described by DUCELLIER.[14] An annual rainfall of over 60 cm., well distributed throughout the year, with a maximum in November and December, and a minimum in July and August, together with a mean temperature ranging from 5° C. in January to 30° C. in August, produces an evergreen vegetation with hardly a cessation of flowers throughout the year. Three distinct bands of vegetation correspond to three distinct topographic zones running parallel with the shore. First is the foredune, with a vegetation characterized by the abundance of annuals and grasses of the usual type, belonging to such well known genera as *Salsola, Cakile, Silene, Euphorbia,* and *Ammophila.* Within this comes a depression termed "bande humide," apparently the same as the "pannes" of European ecologists. Here the vegetation is a mixture of xerophytes, mesophytes, and such hydrophytic forms as species of *Juncus, Scirpus, Orchis, Typha,* and *Nerium.*

In the inland portion of the area there appear to be few dunes of any considerable size. The plants conspicuous in the fixation of the dunes are *Lotus creticus, Scabiosa rutaefolia,* and *Pistacia Lentiscus,* while the established dunes are occupied by *Olea europea, Pinus halepensis, Phillyrea media,* and a considerable number of shrubs and herbs mostly of decidedly xerophytic structure. Among the prominent families represented in the lists of species are the legumes

[14] DUCELLIER L., Étude phytogéographique des dunes de la Baie d'Alger. Rev. Gén. Bot. **23**: 273–308; 321–339. 1911.

with 43 species, the composites with 42 species, the grasses with 55 species, the euphorbias with 7 species, and the orchids with 11 species.—GEO. D. FULLER.

Cuscuta and its host.—Investigating the relations existing between certain species of *Cuscuta* and various hosts, particularly with regard to the connection established between the phloem of parasite and that of the host, THODAY[15] concludes that the cell walls of the haustorial phloem degenerate, and are absorbed at the point of contact with the sieve plates of the host, and the naked protoplasm of the parasite applies itself to the sieve area of the host. No connecting threads of protoplasm are found, and the translocation of food substances appears to be by a passive filtration of the contents of the sieve tubes of the host, forced by internal pressure, escaping into the parasite. This and other evidence favors the conclusion that connecting threads of protoplasm occur only between genetically connected cells. The interpretation of the results contains glaring examples of teleology, as we are assured "that the parasite takes much trouble to make use of the host sieve fields as they are, and not to disturb the mechanics of the sieve tubes"!—GEO. D. FULLER.

The prairies.—Studying the prairies of Iowa, SHIMEK[16] concludes that they were originally covered with floras of six more or less distinct types, and gives lists of species for each. He reviews carefully the various theories as to the factors causing their development, and gives a rather extensive bibliography of the origin of this type of vegetation, with brief notes on many of the titles. His principal contribution consists in attempts to obtain quantitative determinations of certain of the factors which may have been efficient in causing prairie development. Conspicuous among the data obtained are those of the comparative rates of evaporation at prairie and forest stations of observation. These data, although very scanty, seem to be significant, and lead to the conculsion that "exposure to evaporation as determined by temperature, wind, and topography is the primary cause of the treelessness of the prairies."—GEO. D. FULLER.

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[15] THODAY, MARY G. (SYKES), On the histological relations between *Cuscuta* and its host. Ann. Botany **35**: 655–682. 1911.

[16] SHIMEK, B., The prairies. State Univ. Iowa, Lab. Nat. Hist. Bull. **61**: 69–240. *pls. 13*. 1911.

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